Conflicting effects of exercise on the establishment of a short-photoperiod phenotype in Syrian hamster

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Abstract

In the Syrian hamster, winter seasonal inhibition of reproduction occurs in response to decreasing day-length. This inhibitory response is modulated by nonphotic cues. In particular, access to a running-wheel has been shown to produce incomplete gonadal regression. The present study sought to determine whether this occurs as a consequence of wheel-effect on adaptation of the circadian system to short days, or whether downstream physiological responses are involved. Short day adaptation of the circadian clock, which is located in the suprachiasmatic nucleus of the hypothalamus (SCN), was tested using the lengthening of the photosensitive phase of the SCN (assayed by the light-induced c-Fos expression in the SCN) as parameter. We found that wheel-running activity does not inhibit the integration of the photoperiodic change by the SCN even if complete testicular regression is prevented. Moreover, this exercise was even capable of accelerating the lengthening of the photosensitive phase after the transfer to short day-length. Thus, even though wheel-running activity inhibits the short photoperiod induced gonadal regression, it acts on the SCN to accelerate the integration of the photoperiodic change by the biological clock.
Introduction

Many mammals exhibit annual cycles of reproduction and other physiological traits such as body mass and thermogenic capacity. Synchronization of these seasonal adaptations enables animals to maximize their reproductive potential to the annual variation of the environment (i.e. to anticipate them) and to enhance survival during winter periods. At temperate latitudes, annual changes in day-length constitute the main factor through which this synchronization is achieved. Changes of day-length are encoded by the main endogenous circadian clock located in the suprachiasmatic nucleus (SCN) of the hypothalamus (25, 28, 29, 37, 49, 52, 55, 56). The photoperiodic message is transmitted from the SCN to the pineal gland by a polyneuronal pathway and transduced into an endocrine message, the nocturnal secretion of melatonin. Duration of this nocturnal secretion is correlated to the length of the night (5, 57) and photoperiodic variation in melatonin secretion controls seasonal physiological functions (for review, 1, 15, 53).

In addition, a range of nonphotic factors such as nutrition and temperature modulate seasonal photoperiodic responses in Rodents (41) and in Ongulates (13). In the Syrian hamster, provision of a running-wheel leads to spontaneous extended bouts of locomotor activity during the night, and it has been shown that it can reverse, reduce or retard testicular regression (12, 14) and anestrous (7). Moreover, another photoperiodic behavior, the hibernation cycle, is also prevented when hamsters have free access to a wheel (27). Some arguments suggest that wheel-running activity acts, at least in part, by mechanisms other than altering melatonin secretion (21, 39). However, it remains unclear if exercise acts on the SCN to inhibit the integration of the photoperiodic change by the biological clock. The purpose of the present study is to determine if exercise is involved in the integration of the photoperiodic message by the SCN.
In order to determine day-length integration by the SCN, we used expression of light-induced c-Fos protein as a marker for the photosensitive phase of the SCN. This photosensitive phase of the SCN is tied to the length of the night in rats (49) and hamsters (56). Indeed, as already described in golden hamsters transferred from a long photoperiod (LP; LD14:10) to a short photoperiod (SP; LD10:14), a light stimulation applied 13 hours after the dark onset does not immediately induce c-Fos expression within the SCN. Approximately 4 weeks are needed to achieve a complete lengthening of the photosensitive phase.

The data presented indicate that wheel-running does not prevent establishment of a short days photoperiodic state in the SCN, at least as defined by induction of c-Fos protein by light. Rather, it appears that incomplete regression depends on the effects of the running-wheel on downstream metabolic physiology.
Materials and Methods

Animals

Adult male Syrian hamsters (*Mesocricetus auratus*) were used in all experiments. They were born in our colony (originally purchased from Harlan France, Ganat, France) under a long photoperiod with a light-dark cycle (LD) of 14 h of light and 10 h of dark (14:10; light off at 6:00pm) and weaned at 3 weeks of age. They were 3-5 animals per cage, maintained at 22 ±1 °C until the beginning of the experiment. When adults, some hamsters were transferred to the short photoperiod LD10:14 (light off at 6:00pm; see experimental paradigms for detail).

Under both long and short photoperiod, the light intensity was approximately 200 lux during daytime and a constant dim red light (< 1 lux) was on throughout the experiment. They were given food (UAR 105, U.A.R., Villemoison-sur-orge, France) and water *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 French law. All efforts were made to minimize the suffering and number of animals used.

Experimental paradigms

Experiment 1

Purpose of the first experiment was to determine whether wheel-running activity inhibits the lengthening of the photosensitive phase of the SCN after a photoperiodic change from LP to SP. Details of the procedure to follow this lengthening of the photosensitive phase have been
described by Vuillez et al. (56). Briefly, the procedure is based on the capacity of light to induce c-Fos expression in the SCN when applied at the end of the night. A light pulse administered 13 hours (D+13) after the dark onset (light period in LP and dark period in SP) induces c-Fos expression in the SCN of hamsters exposed to SP but not LP. After a photoperiodic change from LD14:10 to LD10:14 (by expanding the night at the dark/light transition, the light/dark transition remaining unchanged), this D+13 light stimulation will begin to induce c-Fos expression in the SCN only few weeks after the transfer, when the SCN has became adapted to SP. This progressive lengthening characterizes the integration of day-length by the SCN.

Since wheel-running activity might have affected the induction of c-Fos expression after the light stimulation at D+13, we introduced in the experimental protocol a control group of hamsters entrained to the SP for 8 weeks (in which the SP was thus integrated). Some hamsters were then given free access to a wheel and were tested for their ability to exhibit light-induced c-Fos expression after 4 weeks of exercise (after 12 weeks of SP exposure). Thus, any difference of light-induced c-Fos expression between this group and control hamsters exposed to SP for 12 weeks but without a wheel would be interpreted as a direct effect of wheel-running activity on light-induced c-Fos expression.

Experimental procedure to examine the role of wheel-running activity is summarized in figure 1. From an initial group of 60 hamsters exposed to LP, 20 were transferred to SP for 8 weeks and testis regression was checked by palpation. Then these hamsters were housed in individual cages and half of them were equipped with a wheel (group SPSPW) while the other half were not (group SPSP). Of the 40 remaining hamsters exposed to LP, 20 were transferred to SP (group LPSP) and 20 stayed in LP (group LPLP). In each group, 10 hamsters were housed individually with a wheel (LPSPW and LPLPW, respectively) and 10 without one.
(LPSP and LPLP, respectively). All hamsters were then treated to test the lengthening of the photosensitive phase of the SCN using the technique already described (56). After four weeks with or without a wheel, half of the animals in each group were light stimulated (200 lux) for 15 min at D+13. For LPLP animals, light was not turned on after the 10 hours of the night and the hamsters were kept in darkness. Half of the LPLP hamsters were also light stimulated 13 hours after the dark onset. Hamsters were sacrificed one hour after the beginning of the light stimulation at D+14. Brains were taken out and processed for c-Fos immunocytochemistry. Testes, seminal vesicles and epididymal white adipose tissue (EWAT) were taken out and weighed.

**Experiment 2**

In the second experiment, we observed the time course of the lengthening of the photosensitive phase of the SCN in hamsters transferred in SP with or without a wheel. Temporal evolution of the SP-induced testicular regression was also observed. Testis size of hamsters exposed to the LP was measured one week before they were transferred to LD10:14 and animals were sacrificed the day of the transfer (w0 group) or 2, 3, 4, 5, 6 and 8 weeks after the transfer (w2, w3, w4, w5, w6 and w8 groups respectively). In each group, all hamsters were housed individually and on the day of the transfer cages were equipped with a wheel (n = 6) or without one (n = 5). The day of the sacrifice, all hamsters were light stimulated for 15 min (200 lux) 13 hours after the dark onset and sacrificed one hour after the beginning of the light stimulation (D+13 groups). Brains were taken out and processed for c-Fos immunocytochemistry. Testes, seminal vesicles and EWAT were taken out and weighed.
Experiment 3

To determine whether the photosensitive phase had lengthened under SP, as opposed to simply shifting relative to lights off, we also exposed animals to light pulses just after dark onset. Four groups of hamsters were transferred from LP to SP with or without a wheel, and sacrificed after the 4th or the 8th week of SP exposure (n = 5 per group). Hamsters were light stimulated 1 hour after the dark onset and sacrificed one hour after the beginning of the light stimulation (D+1 groups). Brains were taken out and processed for c-Fos immunocytochemistry. Testes, seminal vesicles and EWAT were taken out and weighed.

Immunocytochemistry

Hamsters were deeply anesthetized with pentobarbital sodium 6 % (150 mg/kg, i.p.; Sanofi, Libourne, France). Paired testes, seminal vesicles and EWAT were removed and weighed. Hamsters were then perfused transcardially with 100-150 ml of 0.9 % NaCl followed by 250-300 ml of freshly prepared paraformaldehyde 4 % in 0.1 M phosphate buffer (pH=7.4). Brains were removed, post-fixed for 4-5 h at 4 °C, and finally rinsed into phosphate buffer saline 0.1 M (PBS) at 4°C until immunocytochemistry procedure. Fifty micrometer coronal sections of the SCN were prepared on a vibratome. c-Fos detection (sheep anti c-Fos 1/5000, Sigma Genosys, Cambridge, UK) was performed on SCN sections using the avidin-biotin method with diaminobenzidine as the chromogen. c-Fos labeled cells were blind counted on 4 rostro-caudal levels of the SCN, using a monitoring video coupled to a microscope (Leica). Cells inside the SCN and in the hypothalamic area immediately adjacent to the dorso-lateral boundaries of the nuclei, which have been described to be sensitive to light (54), were
counted. Cells exhibiting clear, distinct and unambiguous nuclei immunolabeling were counted, whether c-Fos-ir cells were densely or more weakly immunostained.

**Testis size measurement**

Size of the testis was measured as previously described (27). Determination of testis volume was made using the equation of an ovoid: \( V = \frac{1}{6}\pi LW^2 \).

**Food intake measurement**

Method for food intake measurement was previously described (27). An initial quantity of food was given to hamsters, and on the measurement day, remaining food was taken and weighed. The difference was reported as food consumption per day.

**Statistical analysis**

Results were expressed as mean ± SEM. The analysis of the data was performed using a two or three way analysis of the variance (ANOVA), followed by pairwise post hoc comparisons with the Tukey test (p < 0.05).
Results

Experiment 1

*c-Fos immunoreactivity*

Photomicrographs and results of the quantification of c-Fos immunolabeling are presented in figure 2.

c-Fos expression in the total SCN was affected by light ($F_{1,48} = 123.5; P < 0.001$) and by photoperiod ($F_{2,48} = 5.26; P < 0.01$). Moreover, a strong interaction was found between light stimulation, wheel-running activity and photoperiodic conditions (photoperiod × wheel × light interaction, $F_{2,48} = 9.22; P < 0.001$). In hamsters that were transferred only one day in SP (LPLP groups), c-Fos expression was low, as expected. This expression occurred mainly in the dorsomedial part of the SCN (Fig. 2A) and was not affected by light and wheel-running activity. In the hamsters that had previously integrated the SP (SPSP groups), light stimulation at the end of night induced a high expression of c-Fos protein in the SCN (Fig. 2A). In contrast, c-Fos expression was minimal when no light stimulation was applied (Fig2A). No effect of wheel-running activity was observed on c-Fos in the SPSP group whether or not animals were light stimulated. Finally, in hamsters that were transferred to short photoperiod for 4 weeks, light stimulation induced a significant increase ($P < 0.01$) in hamsters transferred with a wheel (LPSPW-l) when compared to those transferred without one (LPSP-l) (Fig. 2A). Number of c-Fos-immunoreactive (c-Fos-ir) cells in the SCN of LPSPW-l hamsters was similar to the number of cells in hamsters that had integrated the SP, whereas this number in LPSP-l hamster was similar to those obtained in LPLP group. In hamsters that were not light stimulated, c-Fos expression was minimal in LPSPW-n group whereas this expression in LPSP-n group was closer to LPLP groups (Fig. 2B). Thus, the
number of c-Fos positive cells in the SCN was found to be different if hamsters had a wheel or not when they were transferred from LP to SP. Indeed, the number of c-Fos-ir cells was similar between hamsters transferred in SP with a wheel and those in the SPSP groups, whereas levels of c-Fos immunolabeling in animals transferred in SP without a wheel was more similar to LPLP groups.

The results observed here for the entire SCN were found to be similar whatever the rostro-caudal level of the SCN (data not shown). Indeed, an interaction effect between light stimulation, wheel-running activity and photoperiodic conditions was observed at the 4 levels of the SCN which were considered (i.e. every 100 µm).

Testes, seminal vesicles, epididymal white adipose tissue and food intake

Results are presented on figure 3. Because no effect of light was observed on these parameters, groups were pooled independently to the light stimulation.

In the case of testes mass (Fig. 3), a significant effect of wheel was observed ($F_{2,48} = 59.5; P < 0.001$ and $F_{2,48} = 61.2; P < 0.001$, respectively). A interaction effect of wheel and photoperiod was also found ($F_{2,48} = 6.82; P < 0.01$ and $F_{2,48} = 3.18; P < 0.05$, respectively). In hamsters that were transferred to SP only for one day (LPLP groups), testes mass was high and not affected by the wheel-running activity. When hamsters were transferred to SP for 4 weeks, testes mass decreased only if animals had a free access to the wheel (Fig. 3; $P < 0.01$). In SPSP groups, testicular regression was complete in all animals after 8 weeks of SP exposure. Free access to a running-wheel for 4 additional weeks in SP tended to re-increase testes mass but no statistical differences was found.

Similar results were found for the seminal vesicles (Fig. 3). The SP-induced regression of the seminal vesicles was also higher when hamsters had a wheel in their cages. This decrease was
not complete and a significant difference was observed between LPSPW and SPSP groups (P < 0.01).

Epididymal white adipose tissue (EWAT) mass was also affected by the photoperiod (F_{2,48} = 9.64; P < 0.001; Fig. 3), and the mass decreased when hamsters were transferred in SP. A significant effect of the wheel was observed (F_{1,48} = 16.7; P < 0.001) and EWAT mass was lower when hamsters had a free access to a wheel. No interaction of the photoperiod and the wheel was observed (F_{2,48} = 0.97; P = 0.38). In the 3 photoperiodic conditions, wheel-running activity indeed decreased the EWAT mass in a similar manner.

Finally, photoperiod significantly influenced food intake (F_{2,48} = 43.1; P < 0.001), and food intake decreased when hamsters were transferred to SP. Wheel-running activity increased food intake (F_{1,48} = 49.8; P < 0.001). This increase was similar whatever the photoperiodic condition since no interaction was found between photoperiod and wheel (F_{2,48} = 1.11; P = 0.34).

**Experiment 2**

**c-Fos immunoreactivity**

Wheel-running activity significantly affected the speed of the lengthening of the photosensitive phase after a photoperiodic change from LP to SP, as assessed by the light-induced c-Fos expression in the SCN (Fig. 4A). An interaction was observed between the number of weeks in SP and wheel on the number of c-Fos-ir cells in the SCN (F_{6,72} = 3.09; P < 0.01). In hamsters that were transferred to SP with a wheel, the light stimulation 13 hours after the dark onset induced maximal levels of c-Fos expression 2 weeks after the transfer, whereas 4 weeks were needed for hamsters transferred in SP without a wheel.
Testes, seminal vesicles, epididymal white adipose tissue, body mass and food intake

Testes mass was affected by the time in SP (F6, 99 = 8.14; P < 0.001). Moreover, an interaction effect between the wheel and number of weeks spent in SP was observed (F6, 99 = 3.73; P < 0.01). Indeed, hamsters transferred in SP with a wheel exhibited advanced testicular regression when compared to the hamsters transferred in SP without one, even if this testicular regression was not complete after 8 weeks of SP exposure. In hamsters transferred without a wheel, testes mass was minimal 8 weeks after the transfer and characteristic of a total SP-induced testicular regression.

A similar observation is seen when the data are expressed as testicular volume, which was calculated by comparing for each hamster the volume of the left testis when the animal was killed and the volume of the same testis before the transfer in SP. An effect of the time in SP (F6, 99 = 3.81; P < 0.01) as well as an interaction effect (wheel × number of weeks in SP: F6, 99 = 3.72; P < 0.01) was indeed observed.

Seminal vesicles mass was affected, like the testes mass, by the time in SP (F6, 99 = 6.34; P < 0.001) and an interaction effect of the wheel and number of weeks spent in SP (F6, 99 = 2.87; P < 0.05) was also observed. However, EWAT mass was not affected by the time in SP, and no interaction effect was observed. Wheel-running activity however decreased the EWAT mass (F1, 99 = 4.97; P < 0.05).

Variation of body mass was calculated for 8 weeks after the transfer in SP, in hamsters transferred with or without a wheel (n=14 for hamsters with a wheel and n=13 for hamsters without one). Wheel-running activity significantly increased the body mass (F10, 274 = 24.0; P < 0.001), especially after 4 weeks of SP exposure. An interaction effect was indeed found (wheel × number of weeks in SP: F10, 274= 4.05; P < 0.001), and body mass was significantly higher after 6, 7 and 8 weeks of SP exposure in hamsters with a wheel when compared to animals without one (increase of about 20%).
Food intake was affected by photoperiodic change and decreased with time spent in SP ($F_{8, 225} = 21.1$; $P < 0.001$). In addition, wheel-running activity strongly increased food intake (increase of about 20-25 %; $F_{8, 225} = 560.8$; $P < 0.001$), but only after 2 weeks of SP exposure (wheel $\times$ number of weeks in SP interaction: $F_{8, 225} = 22.0$; $P < 0.001$)

**Experiment 3**

In order to ensure that the photosensitive phase lengthened rather than shifted after the transfer to SP, c-Fos immunodetection was assessed after a light stimulation at the beginning of the night (i.e. D+1). In this case, the number of c-Fos-ir cells was lower compared to the number of c-Fos positive cells after a light stimulation at the end of the night (Fig. 6A-B). This was mainly due to the lack of immunolabeling in the dorso-medial part of the SCN. c-Fos expression after the light stimulation at the beginning of the night was higher in the SCN 4 weeks after the transfer in SP compared to 8 weeks (effect of the number of weeks of SP exposure; $F_{1,17} = 33.9$; $P < 0.001$), and free access to the wheel increased c-Fos expression (effect of wheel; $F_{1,17} = 6.15$; $P < 0.05$). No interaction effect was detected.
**Discussion**

In the male Syrian hamster, exposure to short day length induces testicular regression. This SP-induced phenomenon is partially inhibited when animals have a free access to a wheel. Using the lengthening of the photosensitive phase to follow the integration of the SP at the level of the SCN, we have shown that exercise does not inhibit the integration of the photoperiodic change at the level of the biological clock. To the contrary, wheel-running activity accelerates the lengthening of the photosensitive phase of the SCN.

Expression of the immediate-early gene *c-fos* has been extensively used to correlate SCN activity to physiological and behavioral rhythms (for review, 9, 17, 24, 38). For example, photic phase resetting which characterize the photic synchronization of the master circadian clock are correlated to the expression of c-Fos in the SCN. In constant darkness, a light pulse administered during the subjective night shifts the phase of behavioral rhythms and evokes the expression of immediate early genes in the SCN, whereas light has no effect during the subjective day on both phase resetting and immediate early gene expression (22, 23, 33, 42). This light-induced c-Fos expression is mainly restricted to the ventrolateral part of the SCN. By contrast, spontaneous c-Fos circadian expression occurs in the dorsomedial part with a low amplitude peak at the beginning of the subjective day (51). Photic induction of c-Fos was also used to demonstrate that the duration of the photosensitive phase of the SCN depends on photoperiod in rats (49) and in Syrian and European hamsters (56). After a photoperiodic change from LP to SP, the photosensitive phase of the SCN does not extend instantaneously and 2 to 4 four weeks are necessary for a complete lengthening of the photosensitive phase (50, 56). In the present study, we confirm this observation at least in hamsters without a wheel. First, animals transferred in SP for only 1 day (i.e. LPLP groups in experiment 1) elicit endogenous c-Fos expression in the dorsomedial part of the SCN, whether a light pulse is
applied 13 hours after the light/dark transition or not. They were thus at the beginning of their subjective day (51) and this explains why the light pulse was ineffective (22, 42). Second, after several weeks of SP exposure, the light pulse administered at the end of the night induces high-elevated c-Fos expression, as is generally observed after a light pulse during the (subjective) night (2, 22, 40, 42). When no light pulse is applied, c-Fos immunoreactivity is barely detectable in the SCN, as was previously shown in animals killed during the night (22, 40, 42). Thus, hamsters that have integrated the SP respond 13 hours after the beginning of the night like if they were in (subjective) night. Hence, transferring hamsters from LD14:10 to LD10:14 would consist for the animals to consider the add of the 4 hours of darkness from a subjective day to a subjective night. We show here that this integration of the 4 hours of supplementary darkness as night can be observed in light-stimulated hamsters (high increase of c-Fos immunoreactivity in the ventrolateral SCN) as well as in control hamsters which received no light (disappearance of c-Fos expression in the dorsomedial part of the SCN).

Moreover, light stimulation at the end of the night induces a high expression of c-Fos in the SCN 2 weeks after the photoperiodic transfer when hamsters had a free access to a wheel, whereas 4 weeks were needed for the hamsters without one. In addition, disappearance of c-Fos expression in the dorsomedial part of the SCN (as shown in figure 2) is totally performed 4 weeks after the photoperiodic transfer when animals had a wheel, whereas it was not when hamsters had no wheel. These effects of wheel-running activity on light-induced c-Fos expression at the end of the night are not due to a shift of the photosensitive phase since a light stimulation at the beginning of the night induces c-Fos expression in the SCN of hamsters having a free access to a wheel. Thus, we can conclude that the wheel-running activity accelerates the integration of the photoperiodic change by the SCN.
Wheel-running activity was also observed to slightly enhance the expression of c-Fos \textit{per se} in the SCN after a light stimulation at the beginning of the night (experiment 3; Fig. 6). This effect however depends on the time when the light stimulation is applied. Light-induced c-Fos expression at the end of the night is indeed not affected by wheel-running activity. This effect might involve the serotonergic innervation coming from the midbrain median raphe, which constitutes one major afferent pathway conveying nonphotic inputs to the SCN (30). In Syrian hamster, serotonin is released in the SCN in a circadian manner with higher level during the night phase (11). Moreover, novelty-induced wheel-running activity during the day induces the release of serotonin in the SCN (11). It is thus conceivable that wheel treatment induces a change of serotonin level in the SCN, thereby affecting as previously shown the light-induced c-Fos expression (31, 45). However, the onset of the light-induced c-Fos expression was not defined in our experiment, leading to the possibility that the effect of the wheel relies more on a different phase angle relative to the lighting condition. In any case, some additional experiments are needed to better understand why/how wheel-running activity affects light-induced c-Fos expression at the beginning of the night.

Wheel-running activity has been extensively studied as a potent nonphotic factor that can interact with photic cues to synchronize the circadian clock (for review, 10, 36). Some studies have strengthened the potential importance of nonphotic effects in influencing the steady-state phase of photic synchronization, (20, 32, 35, 46). Moreover, following jet-lag, re-synchronization to the new light-dark cycle was shown to be accelerated by making the animals active on a single occasion in the middle of their normal rest period, immediately after the shift in the LD cycle (34). One can suppose that following a photoperiodic change, wheel-running activity reinforces zeitgebers by making the animals active during the hours of supplementary night. As a consequence, this information of locomotor activity would in feed-
back act on the SCN to mediate the integration of the new photoperiod. Several effects of
nonphotic factors depend on the NPY projection from the IGL to the SCN (for review, 10). In
addition, IGL neurons are activated when hamsters are given a novel wheel (19), 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 (16) as in vitro (4). Interestingly, it
has been shown that a bilateral IGL lesion delays the integration of a photoperiodic change by
the SCN (18, 26). Locomotor activity might therefore accelerate the integration of a day-
length change by modulating NPY release in the SCN.

The accelerated integration of day-length by the SCN when hamsters have a free access to a
wheel is linked to an advance of the testicular regression. The photoperiodic response of
reproduction is controlled by the hormone melatonin, and lengthening of its secretion induces
gonadal quiescence in Syrian hamsters (for review, 47, 48, 53). One can thus suppose that the
acceleration of the integration by the SCN accelerates, as a consequence, the lengthening of
melatonin secretion and consequently the testicular regression that is seen in the present study
3-4 weeks after the photoperiodic transfer. This accelerated testicular regression when
hamsters have a free access to a wheel is however not complete. This is in agreement with
previous results that described a more or less complete inhibition of SP-induced testicular
regression by exercise (12, 14). This effect of exercise probably depends on other factors
known to be involved in the control of reproduction. Metabolic signals are specially of
interest. Indeed, it is known that metabolic shortage inhibits reproduction whatever the
photoperiod (43; for review, 44). When given free access to a wheel, Syrian hamsters not only
run very long distance (i.e. several kilometers), but also increase their food consumption and,
as a consequence, increase in their body mass (6, 8, 27). Thus, it is possible that these changes
in food consumption and body mass are responsible for the inhibition of the SP-induced testicular regression by the wheel-running activity. These dual effects of the running wheel on the SP-induced testicular regression discussed above are presented on figure 7.

In conclusion, wheel-running accelerates the integration of a photoperiodic change by the SCN, and also tends to initiate earlier the gonadal atrophy associated with short day-length. However, by mechanisms that are not related to the circadian clock, the running wheel also inhibits the complete SP-induced testicular regression (Fig. 7). Further experiments are needed to delineate how this inhibiting effect occurs and whether it is related to energetic metabolism.
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References


Figures legends

Figure 1

Experimental procedure of the experiment 1.
See materials and methods for details.

Figure 2

Effects of wheel-running activity on the integration of a photoperiodic change by the SCN.
Hamsters were exposed to the short photoperiod LD10:14 for only one day (LPLP groups), for 4 weeks (LPSP groups) or for 12 weeks (SPSP groups). Some hamsters had a free access to a running wheel for 4 weeks prior the sacrifice and some hamsters were light stimulated at the end of the night. See material and methods for details.
A: representative photomicrographs of c-Fos immunoreactivity within the SCN. Scale bar =250 µm.
B: Quantification of c-Fos immunoreactivity within the SCN of hamsters exposed to different light-dark conditions, with or without a wheel, and light-stimulated or not (n=5 in each group). Values are mean ± SEM

Figure 3

Effects of wheel-running activity on the integration of the SP.
Testicular mass, seminal vesicles mass, food intake and epididymal white adipose tissue mass of golden hamsters exposed to the long photoperiod LD14:10 (LPLP groups), transferred to
the short photoperiod LD10:14 for 4 weeks (LPSP groups), or exposed for 12 weeks to LD10:14 (SPSP groups). Four weeks before sacrifice, hamster cage was equipped with a wheel or not. Each group corresponds to hamsters (n=10 per group) that were light stimulated at the end of the night (n=5 per group) and to hamsters that were not (n=5) because no effect of light could be detected on the 5 parameters studied (P > 0.05). Values are mean ± SEM. Differences are indicated by columns having no letter in common (P < 0.05).

Figure 4

*Effects of wheel-running on the time-course of the integration of the photoperiod by the SCN.*

Syrian hamsters were exposed to LD14:10 and then transferred for 2, 3, 4, 5, 6 or 8 weeks to LD10:14 with or without a wheel. The day of the sacrifice, animals (n=6 for hamsters with a wheel and n=5 for the hamsters without one) were light stimulated 13 hours after the dark onset for 15 min, and killed one hour later. Values (mean ± SEM) represent the number of c-Fos immunoreactive cells within the SCN. Significant differences are indicated by * (P < 0.05).

Figure 5

*Effects of wheel-running on the time-course of the integration of the photoperiod.*

Syrian hamsters were exposed to LD14:10 and then transferred for 2, 3, 4, 5, 6 or 8 weeks to LD10:14 with a free access to a running wheel (n=6 per group) or without a wheel (n=5 per group). Testicular mass, seminal vesicles mass and epididymal white adipose tissue mass were measured the day of the sacrifice. Body weight and daily food intake were weekly measured on 13 hamsters transferred to LD10:14 without a wheel, and on 14 hamsters
transferred to LD10:14 with a free access to a wheel. Values are mean ± SEM. Significant differences are indicated by * (P < 0.05).

Figure 6

Effects of wheel-running activity on the light-induced c-Fos expression at the beginning of the night within the SCN.

A-B: Syrian hamsters were exposed to LD14:10 and then transferred to LD10:14 for either 4 or 8 weeks with a free access to a running wheel (n=5 per group) or without a wheel (n=5 per group). Hamsters were light-stimulated one hour after the dark onset for 15 min and killed one hour later. This light stimulation at the beginning of the night induced c-Fos expression within the ventrolateral part of the SCN whether the hamsters have a wheel or not (A). The number of c-Fos immunoreactive cells was however less important than after a light stimulation at the end of the night (i.e. 13 hours after the dark onset; B).

C: Number of c-Fos immunoreactive cells within the SCN. Values are mean ± SEM. Differences are indicated by columns having no letter in common (P < 0.05).

Figure 7

Hypothetical model explaining the effects of exercise on the short photoperiod-induced testicular regression in golden hamsters.

When male golden hamster are transferred from a long (LP) to short photoperiod (SP), they undergo a testicular regression which is complete after 8 weeks of SP exposure (A). This testicular regression is however disturbed when hamsters have a free access to a wheel, and wheel-running activity would affect this seasonal adaptation via 2 different mechanisms (B).
Wheel-running activity might first accelerate the testicular regression by mechanisms depending on the biological clock which is located in the suprachiasmatic nucleus (SCN) (Arrow 1). Indeed, the integration of the photoperiodic change by the SCN is accelerated when hamsters have a free access to a wheel. Second, wheel-running activity might disturb the energetic balance. Indeed, the hypothalamo-hypophyso-gonadal axis is linked to the metabolism, and wheel-running activity disturbs it as it was observed for food consumption (see results and discussion). As a result (C), wheel-running activity accelerates but prevents however a complete SP-induced testicular regression.
Figure 2

A

No wheel

<table>
<thead>
<tr>
<th>Light</th>
<th>No light</th>
<th>Light</th>
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</tr>
</thead>
<tbody>
<tr>
<td>LPLP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LPSP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPSP</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

B

Number of c-FOS-ir cells

<table>
<thead>
<tr>
<th>Wheel</th>
<th>Light</th>
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<tbody>
<tr>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
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<tr>
<td>-</td>
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<td>+</td>
<td>-</td>
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<td>-</td>
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</tbody>
</table>

LPLP  LPSP  SPSP
Figure 3

Testicular Mass

<table>
<thead>
<tr>
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<th>-</th>
<th>+</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPLP</td>
<td></td>
<td>a</td>
</tr>
<tr>
<td>LPSP</td>
<td>b</td>
<td></td>
</tr>
<tr>
<td>SPSP</td>
<td></td>
<td>b</td>
</tr>
</tbody>
</table>

Seminal vesicles mass

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<th>+</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
<td>a</td>
</tr>
<tr>
<td>LPSP</td>
<td>b</td>
<td></td>
</tr>
<tr>
<td>SPSP</td>
<td></td>
<td>b</td>
</tr>
</tbody>
</table>

Food intake

<table>
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</thead>
<tbody>
<tr>
<td>LPLP</td>
<td></td>
<td>b</td>
</tr>
<tr>
<td>LPSP</td>
<td>a</td>
<td></td>
</tr>
<tr>
<td>SPSP</td>
<td>a</td>
<td></td>
</tr>
</tbody>
</table>

Epididymal white adipose tissue mass

<table>
<thead>
<tr>
<th></th>
<th>-</th>
<th>+</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPLP</td>
<td>a</td>
<td></td>
</tr>
<tr>
<td>LPSP</td>
<td>b,c</td>
<td>b,c</td>
</tr>
<tr>
<td>SPSP</td>
<td>b,c</td>
<td>c</td>
</tr>
</tbody>
</table>
Figure 4

![Graph showing the number of c-Fos-ir cells over weeks in SP for subjects with and without a wheel.](image-url)
Figure 5

- **Testicular mass (g)**
  - No wheel
  - Wheel

- **Seminal vesicles mass (g)**
  - No wheel
  - Wheel

- **Epididymal white adipose tissue mass (g)**
  - No wheel
  - Wheel

- **Body mass (g)**
  - No wheel
  - Wheel

- **Daily food intake (g)**
  - No wheel
  - Wheel

Weeks in SP
Figure 6
Figure 7

A  Time course of the testicular regression in golden hamster

B  Effects of the wheel-running activity

C  Resulting effects of the wheel-running activity on the time course of the testicular regression in golden hamster