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

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Menet, Jerome S., Patrick Vuillez, Daniel Bonn, Aurore Senser, and Paul Pévet. Conflicting effects of exercise on the establishment of a short-photoperiod phenotype in Syrian hamster. *Am J Physiol Regul Integr Comp Physiol* 288: R234–R242, 2005. First published August 19, 2004; doi:10.1152/ajpregu.00029.2004.—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 (SCN) of the hypothalamus, was tested by lengthening the photosensitive phase of the SCN (assayed by light-induced c-Fos expression in the SCN) as a 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, although 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.

c-*fos*; wheel-running activity

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, see Refs. 1, 15, 53).

In addition, a range of nonphotic factors such as nutrition and temperature modulates 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 this activity 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 the alteration of melatonin secretion (21, 39). However, it remains unclear whether 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 whether exercise is involved in the integration of the photoperiodic message by the SCN.

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 night in rats (52) and in hamsters (56). Indeed, as already described in Golden hamsters transferred from a long photoperiod [LP; 14:10-h light-dark cycle (LD14:10)] to a short photoperiod [SP; 10:14-h light-dark cycle (LD10:14)], a light stimulation applied 13 h after the dark onset does not immediately induce c-Fos expression within the SCN. Approximately 4 wk 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-day 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 LP with a light-dark cycle of 14 h of light and 10 h of dark (lights off at 6:00 PM; LD14:10) and weaned at 3 wk of age. There were three to five animals per cage, maintained at 22 ± 1°C until the beginning of the experiment. When adulthood was reached, some hamsters were transferred to the SP with a 10:14-h light-dark cycle (light off at 6:00 PM; LD10:14; see *Experimental Paradigms* below for details).

Under both LP and SP conditions, the light intensity was ~200 lux during the daytime, with a constant dim red light (<1 lux) on throughout the experiments. Hamsters were given food (UAR 105, U.A.R., Villemoison-sur-orge, France) and water ad libitum.

All experiments were performed in accordance with the National Institutes of Health *Guiding Principles in the Care and Use of Animals* (NIH publication no. 86-23, revised 1985) as well as in

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accordance with French law. All efforts were made to minimize the suffering and number of animals used.

Experimental Paradigms

Experiment 1. The 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 h (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 to LP. After a photoperiodic change from LD14:10 to LD10:14 (by expanding the night at the dark-to-light transition, the light-to-dark transition remaining unchanged), this D+13 light stimulation will begin to induce c-Fos expression in the SCN only a few weeks after the transfer, when the SCN has adapted to SP. This progressive lengthening characterizes the integration of day length by the SCN.

Because wheel-running activity might have affected the induction of c-Fos expression after the light stimulation at D+13, in the experimental protocol, we introduced a control group of hamsters entrained to the SP for 8 wk (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 wk of exercise (after 12 wk of SP exposure). Thus any difference of light-induced c-Fos expression between this group and control hamsters exposed to SP for 12 wk but without a wheel would be interpreted as a direct effect of wheel-running activity on light-induced c-Fos expression.

Experimental procedures to examine the role of wheel-running activity are summarized in Fig. 1. From an initial group of 60 hamsters exposed to LP, 20 were transferred to SP for 8 wk, and testis regression was checked by palpation. These hamsters were then housed in individual cages, with half of them equipped with a wheel (group SPSPW) and the other half not having a wheel (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 4 wk 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 h of the night and the hamsters were kept in darkness. Half of the LPLP hamsters were also light stimulated 13 h after the dark onset. Hamsters were euthanized 1 h 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 1 wk before hamsters were transferred to LD10:14; animals were killed the day of the transfer or 2, 3, 4, 5, 6, and 8 wk after the transfer. 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 death, all hamsters were light stimulated for 15 min (200 lux) 13 h after the dark onset and killed 1 h after light stimulation began (D+13 groups). Brains were taken out and processed for c-Fos immunocytochemistry. Testes, seminal vesicles, and EWAT were removed 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 killed after the 4th or 8th wk of SP exposure ($n = 5$ per group). Hamsters were light stimulated 1 h after the dark onset and killed 1 h after light stimulation began (D+1 groups). Brains were taken and processed for c-Fos immunocytochemistry. Testes, seminal vesicles, and EWAT were removed and weighed.

Immunocytochemistry

Hamsters were deeply anesthetized with 6% pentobarbital sodium (150 mg/kg ip; Sanofi, Libourne, France). Paired testes, seminal

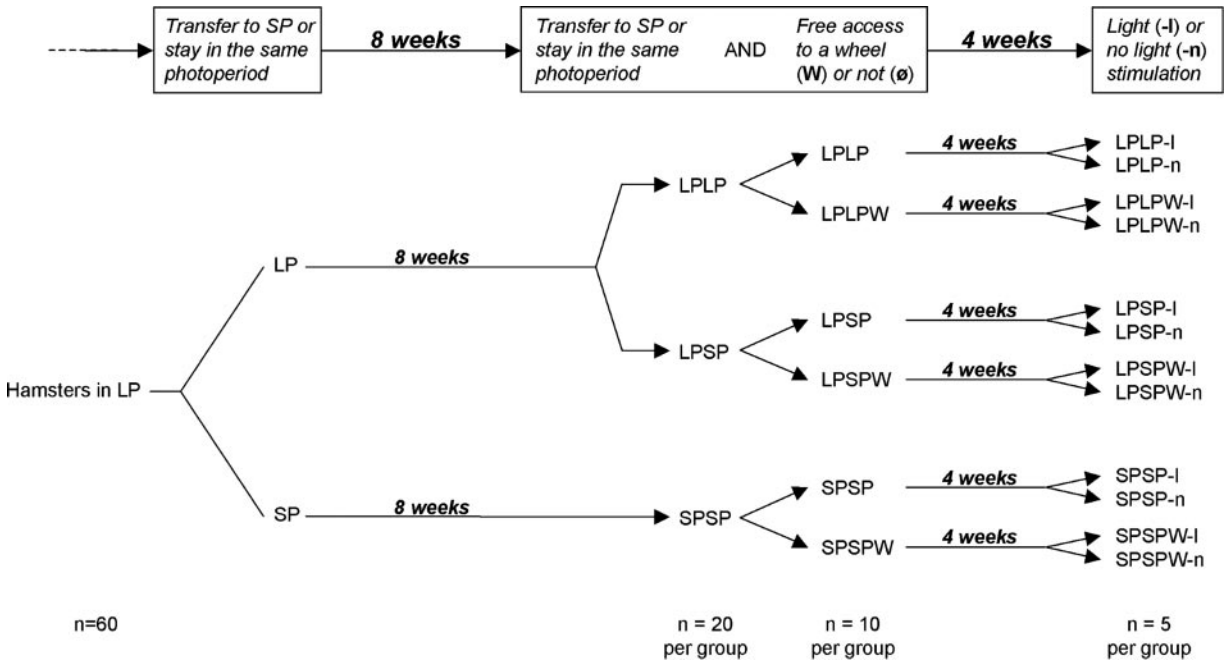

Fig. 1. Experimental procedure of *experiment 1*. See MATERIALS AND METHODS for details and explanation of groups. LP, long photoperiod; SP, short photoperiod.

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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 4% paraformaldehyde 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 1× PBS at 4°C until the immunocytochemistry procedure. Fifty-micrometer coronal sections of the SCN were prepared on a vibratome. We performed c-Fos detection (sheep anti c-Fos 1/5,000; Sigma Genosys, Cambridge, UK) on SCN sections using the avidin-biotin method with diaminobenzidine as the chromogen. c-Fos-labeled cells were blind counted on four rostrocaudal 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 dorsolateral 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-immunoreactive (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 (V) was made with the following ovoid equation: $V = 1/6 \cdot \pi \cdot L \cdot W^2$, where L is length and W is width.

Food Intake Measurement

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

Statistical Analysis

Results are expressed as means ± SE. The analysis of the data was performed using a two- or three-way ANOVA, followed by pairwise post hoc comparisons with Tukey's test ($P < 0.05$).

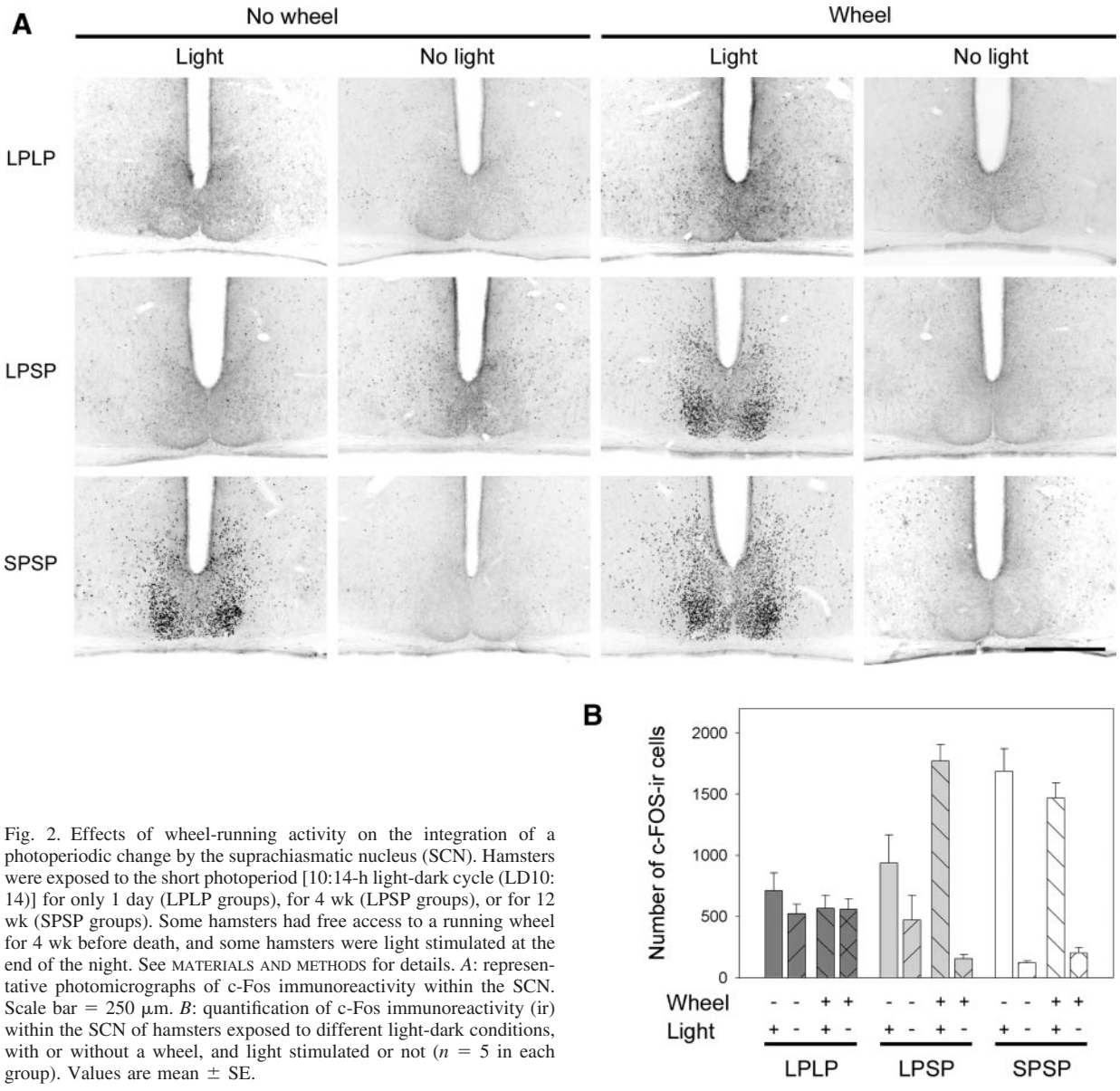

Fig. 2. Effects of wheel-running activity on the integration of a photoperiodic change by the suprachiasmatic nucleus (SCN). Hamsters were exposed to the short photoperiod [10:14-h light-dark cycle (LD10:14)] for only 1 day (LPLP groups), for 4 wk (LPSP groups), or for 12 wk (SPSP groups). Some hamsters had free access to a running wheel for 4 wk before death, and some hamsters were light stimulated at the end of the night. See MATERIALS AND METHODS for details. *A*: representative photomicrographs of c-Fos immunoreactivity within the SCN. Scale bar = 250 μm. *B*: quantification of c-Fos immunoreactivity (ir) 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 ± SE.

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RESULTS

Experiment 1

c-Fos immunoreactivity. Photomicrographs and results of the quantification of c-Fos immunolabeling are presented in Fig. 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 for 1 day in SP (LPLP groups), c-Fos expression was low, as expected. This expression occurred mainly in the dorsomedial part of the SCN (Fig. 2*A*) 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. 2*A*). In contrast, c-Fos expression was minimal when no light stimulation was applied (Fig. 2*A*). No effect of wheel-running activity was observed on c-Fos expression in the SPSP group regardless of light stimulation. Finally, in hamsters that were transferred to SP for 4 wk, light stimulation induced a significant increase ($P < 0.01$) in hamsters transferred with a wheel (LPSPW) compared with those transferred without one (LPSP) (Fig. 2*A*). The number of c-Fos-ir cells in the SCN of light-stimulated LPSPW hamsters was similar to the number of cells in hamsters that had integrated the SP, whereas this number in light-stimulated LPSP hamsters was similar to those obtained in LPLP group. In hamsters that were not light stimulated, c-Fos expression was minimal in LPSPW group, whereas this expression in the LPSP group that was not light stimulated was closer to LPLP groups (Fig. 2*B*). 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 rostrocaudal level of the SCN (data not shown). Indeed, an interactive effect between light stimulation, wheel-running activity, and photoperiodic conditions was observed at the four levels of the SCN considered (i.e., every 100 μm).

Testes, seminal vesicles, EWAT, and food intake. Results are presented in Fig. 3. Because no effect of light was observed on these parameters, groups were pooled independently of 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). An interactive 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 1 day (LPLP groups), testes mass was high and not affected by the wheel-running activity. When hamsters were transferred to SP for 4 wk, 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 wk of SP exposure. Free access to a running wheel for 4 additional wk in SP resulted in a tendency for reincreased testes mass; however, no statistical differences were 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 ($P < 0.01$).

EWAT mass was also affected by the photoperiod ($F_{2,48} = 9.64$, $P < 0.001$; Fig. 3), and the mass decreased when

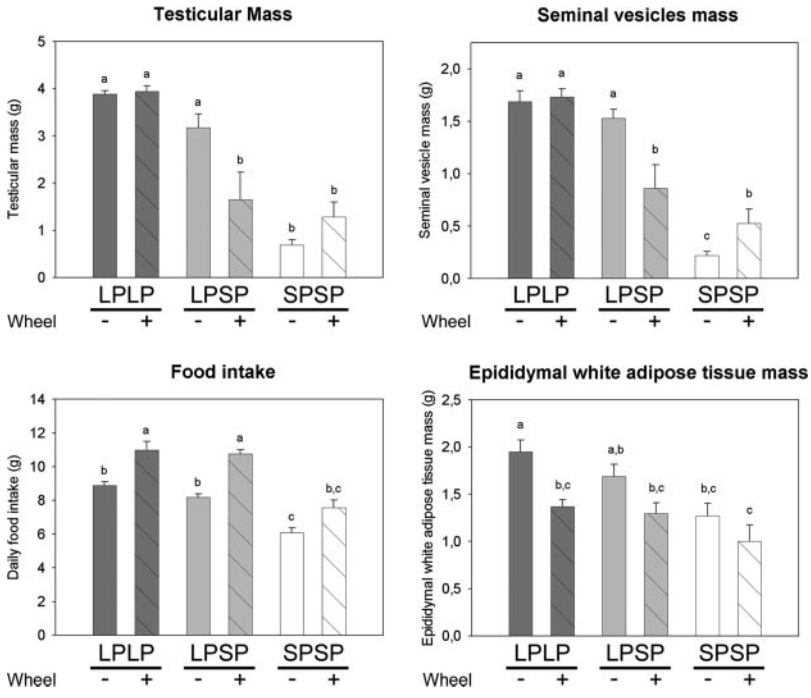

Fig. 3. Effects of wheel-running activity on the integration of the SP. Testicular mass, seminal vesicles mass, food intake, and epididymal white adipose tissue mass of Syrian hamsters were subjected to LD14:10 (LPLP groups), transferred to LD10:14 conditions for 4 wk (LPSP groups), or exposed for 12 wk to LD10:14 (SPSP groups). Four weeks before death, some hamster cages were equipped with a wheel. 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 means ± SE. Differences are indicated by columns having no letter in common ($P < 0.05$).

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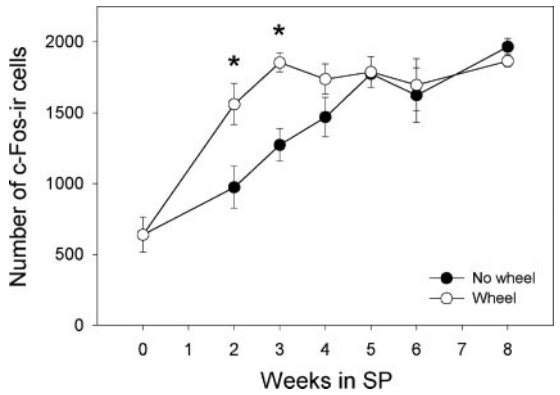

Fig. 4. Effects of wheel running on the time course of the integration of the photoperiod by the SCN. Syrian hamsters were exposed to LD14:10 and then transferred for 2, 3, 4, 5, 6, or 8 wk to LD10:14 with or without a wheel. The day of death, animals ($n = 6$ for hamsters with a wheel and $n = 5$ for the hamsters without one) were light stimulated 13 h after the dark onset for 15 min and killed 1 h later. Values (means ± SE) represent the number of c-Fos-ir cells within the SCN. *Significantly different, $P < 0.05$.

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 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 three 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; 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. 4). An interaction was observed between the number of weeks in SP and wheel presence 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 h after the dark onset induced maximal levels of c-Fos expression 2 wk after the transfer, whereas 4 wk were needed for hamsters transferred in SP without a wheel.

Testes, seminal vesicles, EWAT, body mass, and food intake. Figure 5 summarizes testes, seminal vesicles, EWAT, body mass, and food intake results. Testis mass was affected by the time in SP ($F_{6,99} = 8.14$, $P < 0.001$). Moreover, an interactive effect between the wheel and number of weeks spent in SP was observed ($F_{6,99} = 3.73$, $P < 0.01$). Indeed, hamsters transferred in SP with a wheel exhibited advanced testicular regression compared with hamsters transferred in SP without one,

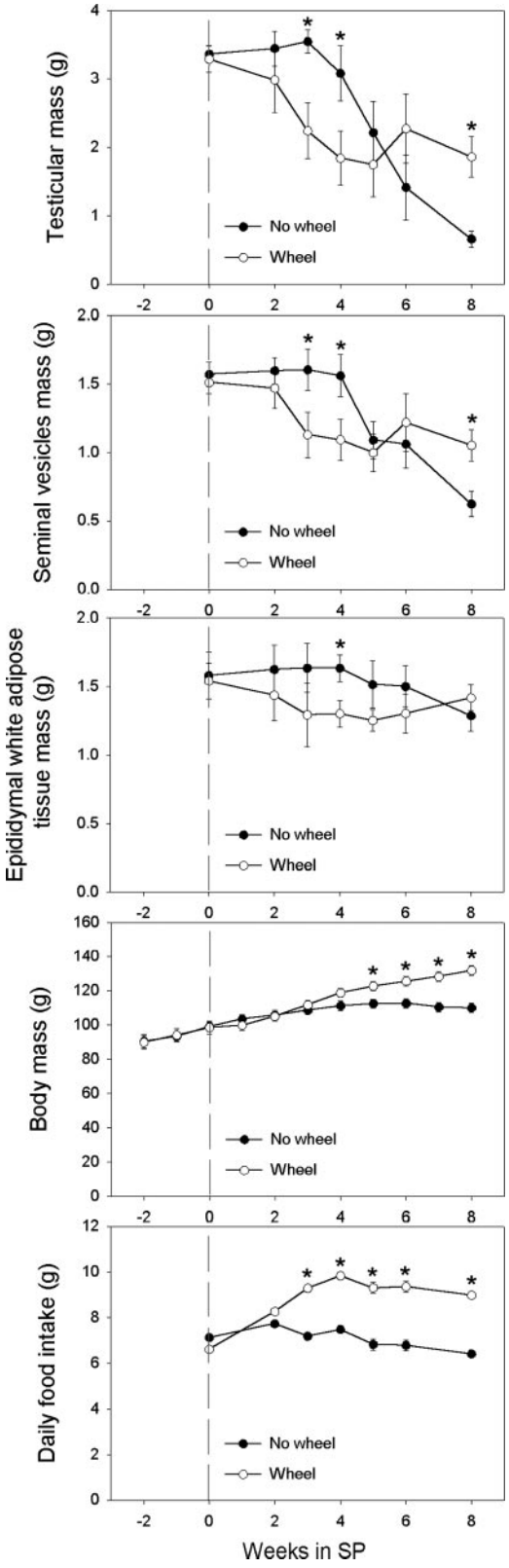

Fig. 5. Effects of wheel running on the time course of the integration of the photoperiod. Syrian hamsters were exposed to LD14:10 and then transferred for 2, 3, 4, 5, 6, or 8 wk to LD10:14 with free access to a running wheel ($n = 6$ per group) or without a wheel ($n = 5$ per group). Testicular mass, seminal vesicles mass, and epididymal white adipose tissue mass were measured the day of death. Body weight and daily food intake were measured weekly on 13 hamsters transferred to LD10:14 without a wheel and on 14 hamsters transferred to LD10:14 with free access to a wheel. Values are means ± SE. *Significantly different, $P < 0.05$.

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even if this testicular regression was not complete after 8 wk of SP exposure. In hamsters transferred without a wheel, testis mass was minimal 8 wk after the transfer, 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 ($F_{6,99} = 3.81$, $P < 0.01$) as well as an interaction effect (wheel × number of weeks in SP: $F_{6,99} = 3.72$, $P < 0.01$) was indeed observed.

Seminal vesicle mass was affected, like the testes mass, by the time in SP ($F_{6,99} = 6.34$, $P < 0.001$), and an interactive effect of the wheel and number of weeks spent in SP ($F_{6,99} = 2.87$, $P < 0.05$) was also observed. However, EWAT mass was not affected by the time in SP, and no interactive effect was observed. Wheel-running activity however decreased the EWAT mass ($F_{1,99} = 4.97$, $P < 0.05$).

Variation of body mass was calculated for 8 wk 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 ($F_{10,274} = 24.0$, $P < 0.001$), especially after 4 wk of SP exposure. An interactive effect was indeed found (wheel × number of weeks in SP: $F_{10,274} = 4.05$, $P < 0.001$), and body mass was significantly higher after 6, 7, and 8 wk of SP exposure in hamsters with a wheel compared with animals without one (increase of ~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 ~20–25%; $F_{8,225} = 560.8$, $P < 0.001$) but only after 2 wk of SP exposure (wheel × number of weeks in SP interaction: $F_{8,225} = 22.0$, $P < 0.001$)

Experiment 3

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 with the number of c-Fos-positive cells after a light stimulation at the end of the night (Fig. 6, *A* and *B*). This was mainly due to the lack of immunolabeling in the dorsomedial part of the SCN. c-Fos expression after the light stimulation at the beginning of the night was higher in the SCN 4 wk after the transfer in SP compared with 8 wk (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 interactive effect was detected.

DISCUSSION

In the male Syrian hamster, exposure to a short day length induces testicular regression. This SP-induced phenomenon is partially inhibited when animals have free access to a running 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. Rather, 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, see Refs. 9, 17, 22, 38). For example, photic phase resetting, which characterizes the photic synchronization of the master circadian clock, is 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 (23, 24, 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 the photoperiod in rats (52) 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 2–4 wk 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*) elicited endogenous c-Fos expression in the dorsomedial part of the SCN, regardless of whether a light pulse is applied 13 h after the light-to-dark transition.

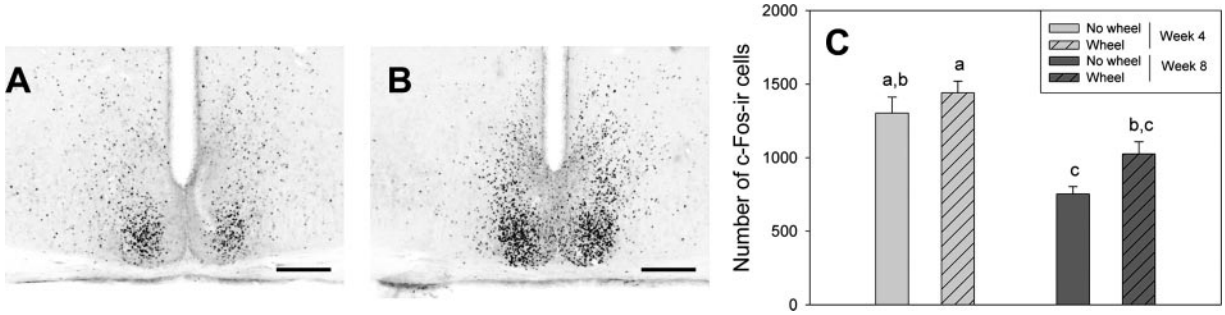

Fig. 6. Effects of wheel-running activity on the light-induced c-Fos expression at the beginning of the night within the SCN. *A* and *B*: Syrian hamsters were exposed to LD14:10 and then transferred to LD10:14 for either 4 or 8 wk with free access to a running wheel ($n = 5$ per group) or without a wheel ($n = 5$ per group). Hamsters were light stimulated 1 h after dark onset for 15 min and killed 1 h later. This light stimulation at the beginning of the night induced c-Fos expression within the ventrolateral part of the SCN whether the hamsters had a wheel or not (*A*). The number of c-Fos-ir cells was however less important than after a light stimulation at the end of the night (i.e., 13 h after the dark onset; *B*). *C*: number of c-Fos-ir cells within the SCN. Values are means ± SE. Differences are indicated by columns having no letter in common ($P < 0.05$).

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They were thus at the beginning of their subjective day (51), and this explains why the light pulse was ineffective (23, 42). Second, after several weeks of SP exposure, the light pulse administered at the end of the night induced high elevated c-Fos expression, as is generally observed after a light pulse during the (subjective) night (2, 23, 40, 42). When no light pulse was applied, c-Fos immunoreactivity was barely detectable in the SCN, as was previously shown in animals killed during the night (23, 40, 42). Thus hamsters that have integrated the SP respond 13 h after the beginning of the night as though they were in (subjective) night. Hence, transferring hamsters from LD14:10 to LD10:14 means the addition of 4 h of darkness as a subjective night and not a subjective day anymore. We show here that this integration of the 4 h 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 that received no light (disappearance of c-Fos expression in the dorsomedial part of the SCN).

Moreover, light stimulation at the end of the night induced a high expression of c-Fos in the SCN 2 wk after the photoperiodic transfer, when hamsters had free access to a wheel, whereas 4 wk were needed for the hamsters without a wheel. In addition, c-Fos expression in the dorsomedial part of the SCN (as shown in Fig. 2) has totally disappeared 4 wk 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 because a light stimulation at the beginning of the night induces c-Fos expression in the SCN of hamsters having 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 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 hamsters, 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 levels 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 and 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, see Refs. 10, 34). Some studies have strengthened the potential importance of nonphotic effects in influencing the steady-state phase of photic synchronization, (20, 32, 35, 46). Moreover, after jet lag, resynchronization to a new light-dark cycle was shown to be accelerated in animals by making them active on a single occasion in the middle of their normal rest period, immediately after the shift in the LD cycle (36). One can suppose that, after a photoperiodic change, wheel-running activity reinforces zeitgebers by making animals active during the hours of supplementary night. As a consequence, this information of locomotor activity would in feedback act on the SCN to mediate the integration of the new photoperiod. Several effects of nonphotic factors depend on the neuropeptide Y (NPY) projection from the intergeniculate leaflet to the SCN (for review, see Ref. 10). In addition, IGL neurons are activated when hamsters are

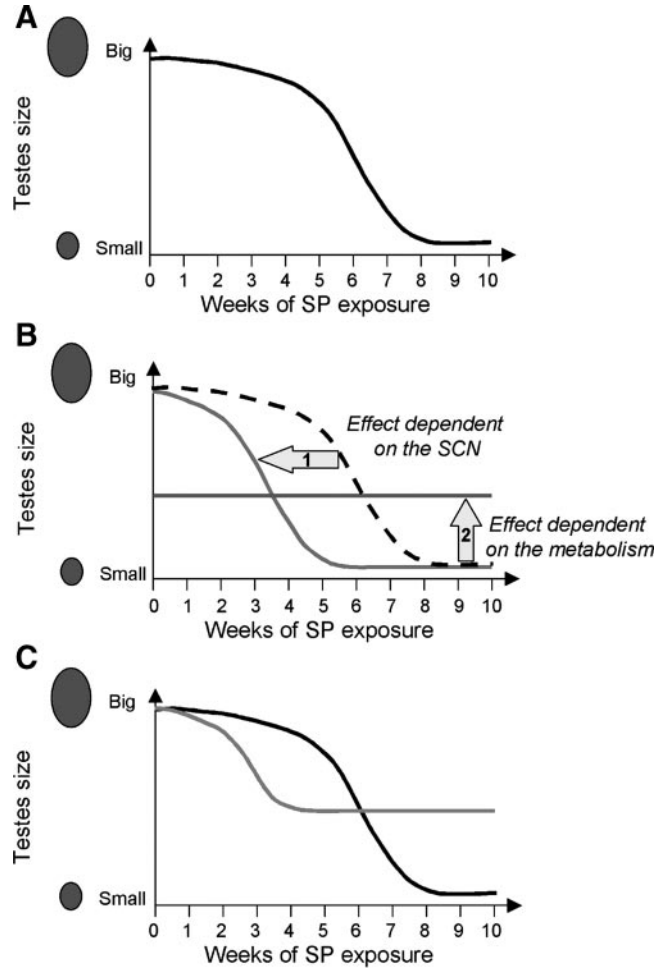

Fig. 7. Hypothetical model explaining the effects of exercise on the short photoperiod-induced testicular regression in Syrian hamsters. When male Syrian hamster are transferred from LP to SP, they undergo a testicular regression that is complete after 8 wk of SP exposure (*A*). This testicular regression is however disturbed when hamsters have 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 that depend on the biological clock, which is located in the SCN (*arrow 1*). Indeed, the integration of the photoperiodic change by the SCN is accelerated when hamsters have free access to a wheel. Second, wheel-running activity might disturb the energetic balance. Indeed, the hypothalamo-hypophyso-gonadal axis is linked to 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.

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given a novel wheel (19); pretreatment with NPY antiserum markedly attenuates phase advances induced by novelty-induced wheel running in hamsters (4), and several effects of nonphotic factors (like behavioral activation) could be mimicked by NPY injections in the SCN in vivo (16) and in vitro (3). 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 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, see Refs. 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 wk after the photoperiodic transfer. This accelerated testicular regression when hamsters have 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 especially of interest. Indeed, it is known that metabolic shortage inhibits reproduction whatever the photoperiod (44; for review, see Ref. 43). When given free access to a wheel, Syrian hamsters not only run very long distances (i.e., several kilometers) but also increase their food consumption and, as a consequence, increase 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 in Fig. 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. Further experiments are needed to delineate how this inhibiting effect occurs and whether it is related to energetic metabolism.

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