Effects of Food Deprivation on Locomotor Activity, Plasma Glucose, and Circadian Clock Resetting in Syrian Hamsters

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Abstract  Circadian rhythms in Syrian hamsters can be phase advanced by activity or arousal stimulated during the daily rest phase (‘subjective day’). A widely used method for stimulating activity is confinement to a novel wheel. Some hamsters decline to run, and some procedures may reduce the probability of running. The authors evaluated food deprivation (FD) as a method to promote running. Given evidence that perturbations of cell metabolism or glucose availability may affect circadian clock function in some tissues or species, they also assessed the effects of FD on free-running circadian phase, resetting responses to photic and nonphotic stimuli and plasma glucose. In constant light, a 27-h fast significantly increased running in a novel wheel and marginally increased the average size of resulting phase shifts. FD, without novel wheel confinement, was associated with some very large phase shifts or disruption of rhythmicity in hamsters that spontaneously ran in their home wheels during the subjective day. Hamsters that ran only during the usual active phase (subjective night) or that were prevented from running did not exhibit phase shifts, despite refeeding in the mid-subjective day. Using an Aschoff Type II design for measuring shifts, a 27-h fast significantly increased the number of hamsters that ran continuously when confined to a novel wheel but did not affect the dose-response relation between the amount of running and the size of the resulting shift. A day of fasting also did not affect the size of phase delay or advance shifts to 30-min light pulses in the subjective night. Plasma glucose was markedly reduced by wheel running in combination with fasting but was increased by running in nonfasted hamsters. These results establish FD as a useful tool for stimulating activity in home cage or novel wheels and indicate that in Syrian hamsters, significant alterations in glucose availability, associated with running, fasting, and refeeding, have surprisingly little effect on circadian pacemaker function.

Key words  circadian rhythms, metabolism, phase shifts, exercise, light pulses

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Circadian rhythms in mammals are entrained (synchronized) to local time primarily by phase and/or period-resetting actions of light on a master circadian pacemaker in the SCN (Weaver, 1998; Aschoff, 1999; Daan, 2000). In several species, regulation of circadian timing by behavioral (so-called nonphotic) stimuli is also well documented. These effects are particularly striking in Syrian hamsters. Behavioral arousal stimulated during the middle of the usual rest phase of the circadian rest-activity cycle (the “subjective day” in nocturnal species) can induce large phase advance shifts, sufficient to modify the phase angle of entrainment to LD cycles, to greatly accelerate reentrainment to a shifted LD cycle and to stably entrain free-running rhythms in the absence of LD cycles (reviewed in Mrosovsky, 1996a; Mistlberger and Skene, 2004). Some of these effects have been observed in other nocturnal and diurnal rodents and in primates, including humans (reviewed in Mistlberger and Skene, 2004, 2005). Although the formal properties of phase shifting and entrainment by arousal have been described in some detail for a few species, a number of neurobiological and functional questions remain to be answered (Mistlberger and Skene, 2004).

The Syrian hamster has proven a valuable model for studies of nonphotic entrainment because of the magnitude of the effects in this species and the precision of its circadian activity rhythms, as measured by spontaneous running in a home cage activity wheel. A convenient method for stimulating arousal during the rest phase in Syrian hamsters is transfer and confinement to a novel wheel; Syrian hamsters typically respond to this procedure by running, and if running is sustained during a 3-h test, phase shifts of 1 to 3 h occur, with only rare exceptions (e.g., Janik and Mrosovsky, 1993). A disadvantage of this technique is that a significant percentage of hamsters may decline to run in the wheel, and those that do not run do not stay awake and do not exhibit phase shifts. In some shipments of hamsters, this so-called sluggard phenotype may characterize 50% or more of the animals (e.g., Janik and Mrosovsky, 1993; Mistlberger et al., 1997; Bobrzynska and Mrosovsky, 1998; Marchant and Morin, 1999). The percentage of “sluggards” may also be increased by brain lesions, gene knockouts, drug treatments, aging, or environmental light, thereby confounding the interpretation of such experiments. Running or arousal can be stimulated by cold exposure or gentle handling, but these methods require special equipment (e.g., refrigeration) and are labor intensive (Janik and Mrosovsky, 1993; Mistlberger et al., 1997; Antle and Mistlberger, 2000).

An alternative method for stimulating activity and arousal in some species is short-term food deprivation (FD). In Syrian hamsters, overnight fasting increases nocturnal home cage wheel activity (e.g., Mistlberger et al., 1997). However, the effects of fasting on the probability or amount of running in a novel wheel during the usual rest period have not been reported. Moreover, this method is not without potential interpretive confounds. Behavioral or metabolic correlates of acute fasting (e.g., hyperactivity, hypoglycemia) or refeeding could shift the clock independently of scheduled activity, producing unpredictable outcomes on circadian phase. These correlates could also modify the response of the clock to nonphotic or photic inputs (for reviews, see Rutter et al., 2002; Challet et al., 2003; Yannielli and Harrington, 2004).

To evaluate acute fasting as a tool for stimulating scheduled activity in Syrian hamsters, we examined the effects of 25.5- to 27-h bouts of FD on the circadian phase in constant dim light (LL) and on the phase-shifting effects of novel wheel confinement in the subjective day and of light pulses in the subjective night. To examine the impact of these manipulations on plasma glucose, blood samples were taken in additional groups tested in parallel. We found that FD does promote both home cage running and running in a novel wheel and that spontaneous or stimulated wheel running during the subjective day can induce very large phase shifts or sudden loss of circadian organization. However, FD does not alter phase-resetting responses to either scheduled running or to nocturnal light pulses, despite inducing marked activity-dependent hypoglycemia.

MATERIALS AND METHODS

Animals and Housing

Young adult, male Syrian golden hamsters (LVG:Lak; ~100 g and 6-8 weeks old at the outset of these studies) were obtained from Charles River, Montreal, PQ. The hamsters were housed individually in polypropylene cages (45 × 25 × 20 cm) equipped with wire mesh floors, running wheels (17.5 cm diameter), and food hoppers for Purina Rodent Chow 5001 pellets. Wheel-running cages
were housed in ventilated isolation cabinets with controlled incandescent lighting (LD 14:10, ~50 lux: 0 lux). Wheel-running activity was detected by microswitches monitored continuously by computer. Activity data were summed and stored to disk at 10-min intervals and periodically transferred to a separate computer for display and analysis.

### Procedures

**Experiment 1: Effects of FD on Circadian Rhythms in Constant Light**

Wheel running and arousal in Syrian hamsters and other nocturnal rodents are inhibited by exposure to light (Aschoff, 1999). To assess whether FD can promote running under conditions that do not favor activity, 3 groups of hamsters were maintained in LL (~25 lux incandescent; Table 1). Group 1 hamsters (n = 18) received a novel wheel confinement test on day 11 of LL (see Fig. 1). The hamsters were gently removed from their home cages and placed in individual Wahmann activity wheels (33 cm) for 3 h, beginning at circadian time (CT) 6 (where, by convention, CT12 is the onset of the daily active period, and each circadian cycle comprises 24 equal circadian hours). The hamsters were then returned to their home cages and left undisturbed for at least 8 days. Phase shifts were measured by comparing regression lines fit to activity onsets for 1 week before and after the wheel confinement test (the so-called Ashoff Type I procedure; Aschoff, 1965). Group 2 hamsters (n = 16) were food deprived for 27 h, beginning at CT6 on days 10 and 23 of LL. During the 1st FD test, the hamsters were confined to a novel wheel for the last 3 h of deprivation (i.e., CT6-9, LL day 11). Ad libitum food access was resumed in the home cage at CT9. During the 2nd FD test, food was removed and returned at
the same CTs, but the animals were not confined to a novel wheel.

Group 2 hamsters were hyperactive in their home wheels when food deprived, and a majority exhibited phase shifts following the 2nd FD test, although they were not confined to a novel wheel during that test. To obtain additional examples and determine if such shifts are dependent on home cage wheel running, rather than FD or recovery feeding per se, group 3 hamsters (n = 20) were fasted 3 times for 27 h beginning on days 10, 23, and 38 of LL. Food was removed at ~CT6 and returned the next circadian cycle at ~CT9. During 1 test (counterbalanced for order), the home cage wheel was locked during FD.

Experiment 2: Effects of FD on Phase Shifts to Stimulated Running

Experiment 1 demonstrated that FD significantly increases wheel running in LL, both in a novel wheel and in the home cage, and that both stimulated and spontaneous home cage running can induce large phase shifts of free-running circadian rhythms, whereas FD without wheel access was not associated with phase shifts. Experiment 2 was designed to assess whether FD affects the dose-response relation between the amount of running during novel wheel confinement and the size of the resulting phase shift. To measure shifts, a modified Aschoff Type II procedure was employed (Aschoff, 1965; Mrosovsky, 1996b). Hamsters (n = 34) were first entrained to LD. On test days, the lights were turned off at zeitgeber time (ZT) 6 (where, by convention, ZT12 is the usual time of lights-off; in LD entrained hamsters, CT12, i.e., the onset of the daily active period, corresponds very closely to ZT12), and the hamsters were confined to a novel running wheel for 3 h (ZT6-9), after which they were returned to the home cage and left undisturbed in constant dark (DD) for the next 4 days (see Fig. 2). Each hamster was subjected to 2 such tests, once during the last 3 h of FD and once without FD. Food was removed at ZT6 on the last day of LD. Ad libitum food access was resumed 27 h later at the end of the novel wheel confinement. Each hamster also received a matching FD test without a light pulse and 2 control DD tests with neither FD nor a light pulse. The 2 control DD tests were conducted at the beginning and end of testing, to assess whether there was a change in the unmasked phase of LD entrainment over the duration of the experiment.

Experiment 3: Effects of FD on Phase Shifts to Light Pulses

An Aschoff Type II procedure was also used to assess the effects of FD on phase shifts induced by 30-min, ~200-lux light pulses provided by cool white fluorescent tubes. Phase shifts induced by this light stimulus were intermediate in size relative to maximal light-induced shifts that we have observed in previous studies, and thus the stimulus was considered nonsaturating and appropriate for detecting modulation by FD. Hamsters (n = 20) were subjected to a series of 4- to 8-day DD tests, each beginning at ZT12 and separated by at least 7 days for reentrainment to LD (see Fig. 4, Table 1). Light pulses were presented at ZT13 (i.e., 1 h after lights-off) to assess phase delay shifts and at ZT20 (8 h after lights-off) to assess phase advance shifts. Light pulses at each ZT were tested twice, once with food ad libitum and once during a 25.5-h FD, where food was removed 25 h earlier and restored at the end of the light pulse (i.e., at ZT13.5 or ZT20.5). Each hamster also received a matching FD test without a light pulse and 2 control DD tests with neither FD nor a light pulse. The 2 control DD tests were conducted at the beginning and end of testing, to assess whether there was a change in the unmasked phase of LD entrainment over the duration of the experiment.

Experiment 4: Effects of FD and Stimulated Running on Plasma Glucose

Plasma glucose was measured in separate groups of hamsters under the following conditions (Table 1): 1) 27-h FD ending at ZT9, 2) 27-h FD and 3-h wheel confinement ending at ZT9, 3) 3-h wheel confinement ending at ZT9, 4) 25.5-h FD ending at ZT13.5 or ZT20.5, and 5) ad libitum fed controls sampled at ZT9, ZT13.5, or ZT20.5. At the designated time, the hamsters were removed individually from the testing room, rendered unconscious via brief exposure to CO₂, and decapitated for collection of trunk blood into chilled heparinized tubes. Blood samples were placed on ice and spun at 3600 rpm for 10 min. Plasma was then extracted and promptly assayed or stored at −80 °C for subsequent analysis. Glucose was measured using the glucose oxidase method (Glucose Color Reagent, Raichem, San Diego, CA) on triplicate 5-μL samples using a microplate spectrophotometer.
Activity data were quantified and plotted on a Macintosh computer using Circadia (Dr. T. A. Houpt, Florida State University) and Prism 4 (GraphPad Software, 2004). The onset of daily wheel running was used to measure phase shifts. Onsets were typically defined as the first 10-min bin with > 50 wheel revolutions after at least 3 h below 50 revolutions in each bin (lower cutoffs were used for some hamsters that were less active). In experiment 1, separate regression lines were fit to at least 1 week of onsets before and after the test days; the displacement of the 2 lines on the day following the test was taken as the phase shift in minutes. In experiments 2 and 3, the average time of activity onset in LD prior to the test day was compared to the time of activity onset on day 2 of DD following the test day. This modified Aschoff Type II procedure is widely used and produces results essentially the same as the Type I procedure, particularly in animals, such as the Syrian hamster, that express very precise circadian rhythms (see Mrosovsky, 1996b). In experiment 3, day 6 was used for the ZT20 light pulse test because of the presence of transient cycles that, if used, would underestimate the final rhythm rapidly decomposed over the next few cycles (e.g., Fig. 1H).

RESULTS

Experiment 1a: FD Stimulates Running in Home and Novel Wheels

Group 1 hamsters ($n = 18$) were subjected to a 3-h novel wheel confinement test in LL without FD. These hamsters accumulated 2966 to 4579 revolutions during wheel confinement, averaging 3592 ± 497 in 3 h. In 16 of 18 cases, wheel confinement induced a phase advance shift, ranging in magnitude from 30 to 220 min (mean = 97 ± 48 min; e.g., Fig. 1A).

Group 2 hamsters ($n = 16$) were fasted twice, on days 10 and 23 of LL. During ad libitum food access prior to each FD day, these hamsters averaged 4213 ± 2642 rev/day (LL days 5-9) and 1688 ± 1969 rev/day (LL days 18-22), respectively ($p < 0.001$; running decreased over time in 15 of 16 cases). During FD, wheel running markedly increased in all 16 hamsters, to 15,001 ± 6935 revolutions during test 1 (24 h total, excluding wheel confinement test) and 16,281 ± 7101 revolutions during test 2 (27 h total). During the last 3 h of the 1st deprivation test, all 16 hamsters ran “continuously” (i.e., at least 1 revolution in each 10-min bin) when confined to a novel wheel, accumulating an additional 3716 to 5588 revolutions, averaging 4618 ± 462 in 3 h, or 28% more than were expressed by ad libitum fed group 1 hamsters during wheel confinement ($p < 0.0001$). The wheel confinement procedure was associated with a phase advance shift in 12 of 16 cases, ranging in magnitude from 30 to 270 min and averaging 132 ± 77 min ($p = 0.046$ by comparison with group 1; e.g., Fig. 1B,D,J). In 1 case, no shift occurred; in another case, the shift was very large but ambiguous in direction (Fig. 1F); and in 2 cases, shifts could not be quantified because of severe disruption of the activity rhythm following the wheel confinement (e.g., Fig. 1H).

Experiment 1b: FD Can Phase Shift Circadian Rhythms in LL by Promoting Home Cage Wheel Running during the Subjective Day

During the 2nd FD test, on day 23 of LL, all group 2 hamsters exhibited elevated home cage wheel running throughout the subjective night. Ten hamsters also exhibited prominent bouts of running during the subjective day, and 9 of these hamsters exhibited phase advance shifts of 40 to 250 min (mean = 91 ± 76 min; e.g., Fig. 1C). In the 10th case, a large shift was evident but was ambiguous in direction (Fig. 1K; activity exhibited 3 daily peaks prior to the 2nd FD test; subjective day running during FD may have advanced the leading component and delayed the trailing component). Five hamsters were hyperactive only during the subjective night, and these hamsters exhibited phase shifts of –70 to 30 min (mean = –17 ± 37 min, e.g., Fig. 1E,G). In the 2 remaining cases, there was a great deal of activity throughout FD, but shifts could not be quantified. In 1 of these cases, the activity rhythm was severely disrupted after the 1st FD and apparently restored by the 2nd FD (Fig. 1H,J). In the other case, a large advance occurred, but the rhythm rapidly decomposed over the next few cycles.

Group 3 hamsters ($n = 20$) were subjected to 3 FD tests without wheel confinement. During 2 tests, the home wheel was available, while during 1 test, the home wheel was locked. Circadian phase in 7 hamsters could not be quantified because of inadequate...
precision or stability of free-running rhythms in LL, either before or after the 1st FD. In 5 of these cases, the hamsters were hyperactive throughout the FD day, and this appeared to precipitate a loss of precision or splitting of the daily activity rhythm into 2 components. The remaining 13 hamsters were also hyperactive during the FD tests with the home cage wheel open. However, in these cases, hyperactivity was limited almost entirely to the subjective night or early subjective day, and no large phase shifts were observed (means = −9 ± 35 min and −2 ± 16 min, for FD 1 and 2, respectively; e.g., Fig. 1M,N). When running wheels were locked during FD, again no large phase shifts were observed (mean = −20 ± 27 min; range, −50 to 60 min; e.g., Fig. 1L,O). Thus, in groups 2 and 3, FD without novel wheel confinement was associated with phase advance shifts only in cases where the hamsters exhibited spontaneous wheel running in their home cages during the middle of the subjective day.
Experiment 2: FD Does Not Affect Running-Induced Phase Shifts

The results of experiment 1 indicate that a 27-h FD significantly increases running during confinement in a novel wheel and marginally increases the average size of resulting phase advance shifts. Experiment 2 was designed to further evaluate the impact of FD on the magnitude of activity-induced phase shifts, using a within-subject design, a larger n, and the Aschoff Type II procedure, which should minimize running in the home cage during the light period prior to novel wheel confinement.

FD initiated at ZT6 in LD significantly increased total daily wheel-running activity, primarily at night (e.g., Fig. 2A), but did not induce a significant group mean phase shift (Fig. 3A). By contrast, wheel confinement from ZT6 to 9 was associated with large phase advance shifts in both the ad libitum food access and FD conditions. A scatterplot relating phase shifts to wheel revolutions during wheel confinement reveals that phase advance shifts > 75 min were associated with cumulative 3-h wheel revolutions > 3000 (Fig. 3B). FD increased by 56% the number of hamsters that ran > 3000 revolutions when confined to a novel wheel for 3 h, during fasting or ad libitum food access. Within the cluster of data points corresponding to wheel counts > 3000, the 7 smallest shifts occurred in the FD condition. However, the maximal phase shifts in the FD and ad libitum food access conditions were comparable, and among the 13 hamsters that ran > 3000 revolutions in both the fasting and ad libitum food access tests, the mean phase shifts also did not differ (169 ± 43 min vs. 170 ± 36 min, respectively; Fig. 4A).

Experiment 3: FD Does Not Affect Light-Induced Phase Shifts

Consistent with the results of experiment 2, FD (25.5 h) conducted within an Aschoff Type II procedure for measuring phase shifts substantially increased home cage running primarily at night (e.g., Fig. 4D,G). Average waveforms revealed that the peak level of running attained during the 1st few hours of the night was not increased by FD but that running was substantially elevated for the remainder of the night.
Despite the additional nocturnal activity and a small amount of daytime running, FD did not induce significant phase shifts, regardless of whether fasting was initiated at ZT12 (2 ± 14 min relative to the ad libitum fed condition, p > 0.05) or ZT19 (7 ± 31 min relative to ad libitum fed condition, p > 0.05). FD also did not affect the magnitude of phase shifts to 30-min light pulses at either ZT13 (mean shifts relative to the control conditions = −45 ± 21 min vs. −37 ± 24 min in ad libitum and fasting conditions, respectively; p > 0.05) or ZT20 (67 ± 22 min vs. 53 ± 25 min in ad libitum and fasting conditions, respectively, p > 0.05; e.g., Fig. 4).

Experiment 4: Plasma Glucose Is Strongly Affected by Wheel Running and Fasting

A 27-h fast ending at CT9 had no effect on plasma glucose levels relative to ad libitum fed controls (92.89 ± 5.87 mg/dL vs. 91.5 ± 9.8 mg/dL). However, when a 27-h fast was combined with a 3-h wheel confinement prior to blood sampling, plasma glucose declined by 37% (p < 0.0006). By contrast, a 3-h wheel confinement in ad libitum fed hamsters resulted in a 28% increase in plasma glucose relative to controls (p < 0.003). A 25.5-h fast, ending at ZT13.5 (i.e., 1.5 h after the onset of nocturnal home cage wheel running), was associated with a 22% decrease in plasma glucose, relative to the ad libitum fed group sampled at the same time (p < 0.00003). A 25.5-h fast ending at ZT20.5 (i.e., 8.5 h after the onset of nocturnal activity) was associated with a ~40% decrease in plasma glucose, relative to the ad libitum fed groups sampled at ZT13.5 or ZT9, but no difference relative to ad libitum fed hamsters sampled at ZT20.5 (55.66 ± 6.48 vs. 58.36 ± 6.34 mg/dL); that is, there was a marked circadian variation in plasma glucose, possibly secondary to the circadian rhythm of running activity in the home cage wheel.

DISCUSSION

This study generated several novel observations relevant to the use of FD as a tool for studying nonphotic zeitgebers and as a probe for evaluating the role of plasma glucose availability in circadian clock function. Perhaps most striking were the large phase shifts evident in hamsters that ran substantially in their home cage wheels during the subjective day when food deprived (e.g., Fig. 1C,K). These are the 1st examples, to our knowledge, of phase advance shifts caused by entirely spontaneous running expressed by hamsters in their home cages, without external stimulation, at a circadian phase when they would normally be quiescent. In cases where increased running was limited to the subjective night, circadian phase was remarkably stable, despite comparable 27-h cumulative wheel revolutions and despite recovery feeding initiated in the subjective day at CT9 (e.g., Fig. 1E,G,N). These results indicate that there is nothing special about novelty as a stimulus for inducing nonphotic phase shifts; even spontaneous running in a familiar home wheel is sufficient to reset the circadian clock. FD can therefore be used as a simple method for stimulating...
activity where there is a need to minimize equipment (e.g., novel wheels) or labor (e.g., to transfer hamsters to novel wheels or to keep them awake by handling).

A single day of FD was also an effective stimulus for increasing the probability of running during confinement to a novel wheel in the middle of the usual sleep period. FD did not change the maximal amount of running during a 3-h wheel confinement test, and in hamsters that ran at comparable levels during fasting and nonfasting tests, there was also no difference in the size of the average phase shift. Thus, FD is a convenient method for promoting scheduled running under lighting conditions (i.e., LL) that do not favor activity and may prove similarly effective in animals that are otherwise hypoactive because of experimental manipulations (e.g., drug injections, brain lesions, gene mutations, etc.).

Inspection of the scatterplot illustrating the relationship between novel wheel-induced running and shift magnitude reveals that among all cases in which wheel revolutions exceeded 3000 in 3 h (an apparent threshold for large phase advance shifts with this apparatus), the 7 smallest phase shifts were in the FD condition. Five of these hamsters failed to run and did not shift in the ad libitum fed condition, and thus they are not informative concerning the effect of fasting on running-induced phase shifts. Two of these “sluggard” hamsters are distinguished by the fact that, when food deprived, they ran 5279 and 5965 revolutions during wheel confinement and yet exhibited phase shifts near 0. To accumulate this many counts in 3 h, the hamsters need to make 300 or more revolutions in each 10-min bin, which is near the maximal level of spontaneous home cage running evident at the beginning of the daily active phase (see Fig. 5) and requires virtually continuous waking. Hamsters that run this much in a novel wheel in the subjective day almost invariably phase shift. Nonetheless, a few exceptions have been reported previously in a study of cold-induced running, and in those cases, the hamsters were also sluggards in the control condition (Janik and Mrosovsky, 1993). Thus, there is a small population of hamsters that tend to be sluggards under normal conditions and that fail to exhibit phase shifts when induced to run at high levels by cold exposure or hunger. Such animals may be a valuable resource for understanding individual differences, physiological conditions, and
neural mechanisms that determine circadian pacemaker responses to nonphotic inputs. FD may have utility as a simple method to screen for such animals. FD per se, independent of increased running, had surprisingly little effect on circadian pacemaker phase. Food-deprived hamsters eat immediately when food access is restored (Mistlberger et al., 1997), although they do not exhibit the marked hyperphagia characteristic of food-deprived rats (Silverman and Zucker, 1976; Rowland, 1983). Nonetheless, to the extent that eating is rewarding to a hungry hamster (such animals will certainly perform work for food), these results indicate that neural correlates of natural reward are not sufficient to reset the circadian pacemaker. Reward processes may explain why hamsters run so much in novel and home cage wheels, but nonphotic inputs to the circadian system likely do not signal reward. Rewarding electrical stimulation of the lateral hypothalamus has been reported to induce non-photic-type phase shifts in hamsters, but these shifts may be secondary to non-specific arousal, motor activity, or activation of fibers of passage to the SCN pacemaker or to sources of nonphotic input to the SCN (Cain et al., 2004).

There has been much recent interest in the relationship between circadian clocks and metabolism. The circadian clock generates daily rhythms in metabolism, and in vitro evidence suggests that metabolic signals can regulate transcription of circadian clock genes and may play a role in clock cycling and light-induced resetting (Rutter et al., 2001; Yu et al., 2004). Also, neural activity in SCN slices can be altered by glucose or insulin (Doyle et al., 1995; Hall et al., 1997; Shibata et al., 1986). Evidence for this hypothesis from in vivo studies of mammals is still scant, and what evidence is available is indirect, from systemic manipulations of glucose availability. Three days of FD phase shifted circadian activity rhythms of rats in LL but had no effect in DD (Coleman and Francis, 1991). A more severe deprivation of 7 days’ duration was reported to induce phase shifts in rats in DD, but these shifts are not clearly evident when regression lines rather than cosine fits are used to quantify phase (Challet et al., 1997a; Challet et al., 1997b); in fact, the overall impression from the published figures is one of a surprising degree of phase and period stability despite the severity of the intervention and obvious changes in the waveform of the activity rhythm during FD. In mice, glucoprivation by systemic injections of the antimetabolite 2-deoxyglucose (2-DG) or insulin attenuated phase delay shifts to light early in the subjective night and phase advance shifts to light late in the subjective night (Challet et al., 1999a), although in a 2nd study, delay shifts to light were not affected by the same dose of insulin (Challet et al., 1999b). Conversely, induction of hyperglycemia in mice by streptozotocin (STZ) treatment enhanced phase delays to light but had no effect on phase advances to light (Challet et al., 1999b). Rats treated with STZ exhibited what appears to be an opposite effect, that is, a decrease in SCN neuronal c-fos expression in response to light early in the night (Yamanouchi et al., 1997). Phase shifts and SCN c-fos induction are highly correlated responses to light; thus, either STZ has different effects on SCN function in mice and rats or it dissociates SCN responses to light. In Siberian hamsters, 2-DG injections inhibited light-induced advances and delays, but the same treatment had no effect on Syrian hamsters (Challet et al., 2000). Finally, restricted feeding schedules can entrain or alter photic entrainment of SCN-mediated rhythms or clock genes in rats, mice, or Syrian hamsters (Abe et al., 1989; Mistlberger, 1993; Challet et al., 1996; Challet et al., 1997; Marchant and Mistlberger, 1997; Challet et al., 1998; Castillo et al., 2004; Mendoza et al., 2005). However, acute glucoprivation and restricted feeding schedules have prominent direct effects on arousal and activity, and it may be these behavioral stimuli that mediate changes in pacemaker phase or response to light. These results suggest that while perturbations of glucose availability by FD or pharmacology can alter circadian rhythms in rodents, this may depend on the species, the circadian phase of the treatment, or behavioral correlates of hypoglycemia or hyperglycemia.

The results of the present study appear to dissociate changes in plasma glucose and circadian phase resetting. Running in a novel wheel was associated with a 40% drop in plasma glucose in fasting hamsters and a 22% rise in fed hamsters, but there was no difference in the size of subsequent phase shifts in those cases where running levels were matched in the fed and fasted conditions. Fasting at night was associated with a significant hypoglycemia early in the night, but this had no effect on phase delay shifts to light pulses. This result is consistent with the observation that glucoprivation induced by systemic 2-DG injection does not affect phase delay shifts to light pulses in Syrian hamsters (Challet et al., 2000). Fasting also had no effect on phase advances to light pulses later in the night, but at this circadian phase, both the fed and fasted hamsters exhibited a comparable hypoglycemia relative to nonfasted hamsters early in the night. Whatever the explanation for these
effects of wheel and food access on plasma glucose, the results clearly demonstrate that circadian clock function in Syrian hamsters is not affected by significant variations in plasma glucose availability. It may be that Syrian hamsters are particularly adept at mobilizing lipid resources to power cellular processes in the brain during fasting; additional measures of plasma free fatty acids, leptin, and insulin will be needed to address this. In any case, the results do not provide positive support for the proposal that cell metabolism may regulate pacemaker function.

Several food-deprived hamsters that were hyperactive during the subjective day in LL showed unusually large phase shifts (indicative of type 0 resetting; e.g., Knoch et al., 2004) or sudden loss of amplitude and precision of the home cage wheel-running rhythm (e.g., Fig. 1F,H,K). In 1 interesting case, the activity level and rhythm were markedly attenuated by the 1st deprivation and restored by the second (Fig. 1H,J). Whether such changes reflect suppression and restoration of pacemaker amplitude, comparable to what has been inferred from studies of critically timed bright light exposure in humans (Jewett et al., 1991), is a matter of speculation.

Animals in their natural habitats may experience regular or occasional bouts of FD. Humans may also experience disruptions of food intake following shift-work rotations or transmeridian jet travel, during dieting, or in illness. This raises the reasonable question of whether acute disturbances of feeding can alter the phase of the circadian pacemaker or its response to photic and nonphotic zeitgebers. In Syrian hamsters, FD induces a rapid behavioral response in the form of hyperactivity in running wheels, but independent of this hyperactivity, FD appears not to affect circadian clock genes in the SCN pacemaker, given that fasting and refeeding affected neither circadian rhythm phase nor resetting responses to photic and nonphotic stimuli. These results appear to distinguish Syrian hamsters from mice, in which metabolic challenges, including FD, do have significant effects on pacemaker function. However, the nature of this species difference remains to be clarified. Whether these effects in other species reflect regulation of central circadian clock genes by metabolic stimuli associated with fasting or refeeding, or are secondary to effects upstream from the pacemaker (e.g., on behavioral state or retinal processing of light), also remains to be established. Finally, despite occasional claims that feeding and fasting can be used to minimize jet lag, systematic research in humans (e.g., Krauchi et al., 2002) is still scant.

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