

Research report

Behavioral inhibition of light-induced circadian phase resetting is phase and serotonin dependent

Ralph E. Mistlberger^{*}, Michael C. Antle*Department of Psychology, Simon Fraser University, Burnaby, BC, Canada V5A 1S6*

Accepted 14 October 1997

Abstract

Circadian rhythms in Syrian hamsters can be phase shifted by light exposure during the subjective night and by a bout of wheel running induced during the subjective day. Interactions between photic and behavioral stimuli were examined by comparing phase shifts to 15 min, 50 lux light pulses with and without a bout of running induced by confinement to a novel wheel 30 min prior to and extending through light exposure. Light pulses 6 h after dark onset on the first night of constant dark induced phase advance shifts averaging 80 min. Wheel running attenuated these shifts by 45% on average ($p < 0.01$). Light pulses 1 h or 2.25 h after dark onset induced phase delay shifts averaging 50 min and 20 min, respectively, that were not affected by stimulated running. A significant running response to the novel wheel was evident at all 3 time points, but was greater to wheel confinement at both times early in the night. Stimulated running alone early or late in the night did not produce significant phase shifts. Behavioral attenuation of phase advances to light late in the night was prevented by pretreatment with the general 5HT₁ antagonist metergoline (2 mg/kg i.p.). Metergoline did not significantly attenuate running in novel wheels. These results indicate that modulation of light-induced phase shifts by behavior is phase dependent and may involve direct or indirect actions of serotonin within the circadian system. © 1998 Elsevier Science B.V.

Keywords: Circadian rhythm; Nonphotic zeitgeber; Wheel running; Metergoline; Syrian hamster

1. Introduction

The principle entraining stimulus (zeitgeber) for circadian rhythms in most species is the daily cycle of light and dark (LD). However, circadian rhythms in some species can also be affected by nonphotic stimuli, including daily cycles of feeding, temperature social interactions and behavioral activity or arousal (reviewed in Refs. [25,26,29,39]). The effects of behavior on circadian rhythms have attracted considerable recent attention. Scheduled running in rodents can phase shift or entrain circadian rhythms [10,20,24,35] and spontaneous running in home cage wheels can modulate both the circadian period [12,42] and waveform [41]. This raises the issue of whether behavioral activity plays a significant role in setting the phase of circadian rhythms in the presence of natural LD zeitgebers. The available evidence suggests that it may; daily or single bouts of stimulated running can alter the phase angle of entrainment to LD cycles [17,38]

and can influence the rate at which rhythms reentrain to a shifted LD cycle [16,30].

Other studies indicate that interactions between behavioral and photic stimuli may be complex. One set of observations indicates that at certain phases light can inhibit nonphotic inputs to the circadian pacemaker (the suprachiasmatic nuclei, SCN). During the mid-subjective day (the rest phase in nocturnal rodents), scheduled activity can induce phase advance shifts of circadian rhythms, and light can block these shifts, despite having no independent shifting effect at this phase [28]. Transmitters involved in nonphotic phase shifting include neuropeptide Y (NPY) and possibly also serotonin (5HT) [5,11,21,29]. Injections of NPY or 5HT agonists during the subjective day induce activity-like phase shifts, and these shifts can be blocked by a subsequent light pulse [6,31]. In vitro, phase shifts of SCN neural activity induced by NPY can be blocked by glutamate [4], which is the most likely neurochemical mediator of photic inputs to the SCN [9].

Another set of observations indicates that at other circadian phases, nonphotic stimuli may inhibit photic inputs to the pacemaker. Phase shifts to light during the subjective night (the active phase in nocturnal rodents) can be attenu-

^{*} Corresponding author. Fax: +1-604-291-3462; E-mail: mistlber@sfu.ca

ated by NPY or 5HT agonists [14,34,40] or potentiated by antagonists to NPY or to 5HT [6,33], and phase shifts to glutamate in vitro can be blocked by NPY [4]. In addition, it has been reported in scorpions that behavioral activity at night can block the normal phase shift response to light pulses [15].

A similar behavioral result has been reported in one mammalian species; hamsters that were spontaneously active or induced to run by confinement to a novel running wheel cage during a light pulse scheduled late in the night showed a significant reduction in the size of the phase advance shift to the photic stimulus [32]. However, the between-group design of that study raises interpretive ambiguities. Animals were divided post hoc into ‘runners’ and ‘non-runners’, and runners were found to exhibit smaller shifts to light. Light inhibits activity in nocturnal rodents [2], including hamsters. Thus, it is conceivable that ‘runners’ ran more and shifted less because they were less sensitive to light or received a weaker photic stimulus due to cage positioning or other factors. A within-subjects design is necessary to rule this out. In addition, subjects differed in the phase of light exposure, and, in at least part of the data set (experiment 1), there is a significant unreported correlation between the size of the phase shifts and the phase of light exposure ($r = 0.57$, $p < 0.001$, calculated from raw data provided in Fig. 1 [32]). Runners and non-runners were not equally distributed across these phases, thus group differences in shift size could be secondary to group differences in phase of light exposure.

Behavioral modulation of photic phase control of circadian rhythms may be of considerable practical importance. Therefore, the aims of the present study were to establish more clearly that wheel running in hamsters can alter phase shifts induced by brief light exposure late in the night and to extend these observations to include early night. In addition, given the evidence that behavioral activity is associated with 5HT release [8,18,37], and that 5HT can antagonize pacemaker responses to light [13,14,34,36], a final aim was to evaluate a role for 5HT in modulation of light-induced phase resetting by behavior.

2. Materials and methods

2.1. Procedures

Adult male Syrian hamsters (Charles River, Montreal) were housed individually in opaque plastic cages (45 × 25 × 20 cm) with wire covers and floors and 17 cm running wheels. The cages were housed in isolation cabinets with controlled incandescent lighting and ventilation fans. Wheel revolutions were detected by microswitch closures monitored continuously by computer using the Activity Counting System interface and software (Simon Fraser University). Phase shifts in response to light, running activity and the combination were assessed using the Aschoff Type II

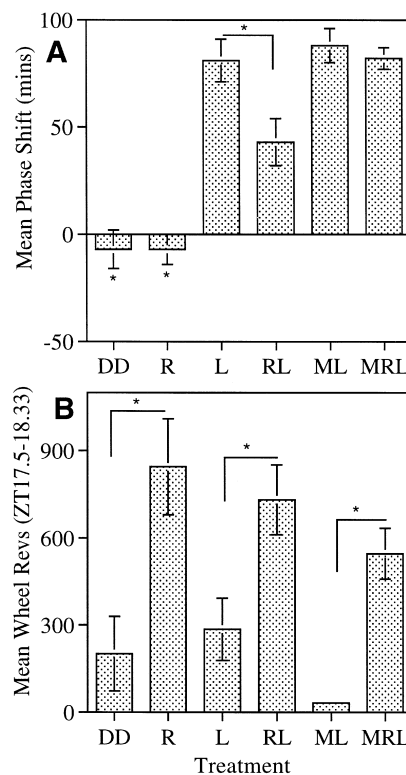


Fig. 1. (A) Group mean phase shifts $\pm$ S.E. in response to constant dark (DD), a 45 min bout of wheel running from ZT17.5–18.25 (R), a 15-min light pulse at ZT18 (L), the combination of wheel running and a light pulse (RL), a light pulse following metergoline administration (ML), and wheel running combined with light following metergoline (MRL). The mean for the DD condition represents the phase shift with respect to LD, while the other means represent the phase shifts adjusted by subtracting these DD shifts. (B) Group mean wheel revolutions from ZT17.5–18.33 (50 min) in each of the treatment conditions. * Denotes significant difference between pairs ($p < 0.05$). In panel A, the values for A, L, RL, ML and MRL are all significantly different from DD and R ($p < 0.05$).

procedure [1], as follows: Hamsters were first maintained in a 14:10 LD cycle (50 lux:0 lux) for at least 10 days and were then exposed to constant dark (DD) for 5–10 days, beginning at the usual time of dark onset. On the first night of DD, the hamsters received a light pulse (15 min, 50 lux), or were induced to run for 45 min by transfer to a novel 17 cm running wheel, or received both treatments, with the light pulse occurring during the last 15 min of the induced running bout. The novel wheels were mounted in plastic bins with sawdust floors, and were placed in the same cabinet position as the home cage, to ensure the same light levels across light pulse tests. Cage transfers were performed with the aid of an infrared viewer. The animals were then left undisturbed for the remaining days in DD. To quantify a phase shift associated with each treatment, the phase of activity onset during DD was compared with the average onset of activity during the last 5 days of LD.

Three groups of hamsters were tested. Group 1 hamsters ($N = 16$) were subjected to the following sequence of treatments; (1) a control DD test (no light pulse or activity

stimulus), counterbalanced for order with (2) a 45 min bout of running induced at zeitgeber time (ZT) 17.5 (5.5 h after dark onset, which is designated ZT12 by convention), (3) a 15-min light pulse beginning at ZT18, counterbalanced with (4) a bout of running combined with a light pulse, (5) an injection of metergoline (Research Biochemicals International, 2 mg/kg, i.p., in DMSO vehicle with a concentration of 2 mg/ml) at ZT17, followed by a light pulse at ZT18, counterbalanced with (6) an injection of metergoline followed by the combination of a running bout and a light pulse. Previous observations indicate that metergoline alone does not alter circadian phase (Antle and Mistlberger, unpublished data) or pacemaker responses to light [14]. DD tests lasted 10 days in each case, and were separated by 10 days in LD to ensure that the hamsters were reentrained prior to the next test.

The second group of hamsters ($N = 19$) was subjected to (1) a light pulse at ZT14.25 counterbalanced with (2) a bout of running at ZT13.75 combined with a light pulse at ZT14.25, (3) a control DD test, (4) a light pulse at ZT13, counterbalanced with (5) a running bout at ZT12.5 with a light pulse at ZT13. A third group of age-matched hamsters ($N = 33$) were used to assess the effects of induced wheel running or wheel-locking at ZT13.75–14.5. DD tests lasted for 5 days and were followed by 10 days in LD for reentrainment.

2.2. Data analyses

Activity data were summed and stored to disc at 10 min intervals, and were inspected visually in the form of standard ‘actograms’. Phase shifts were quantified by a

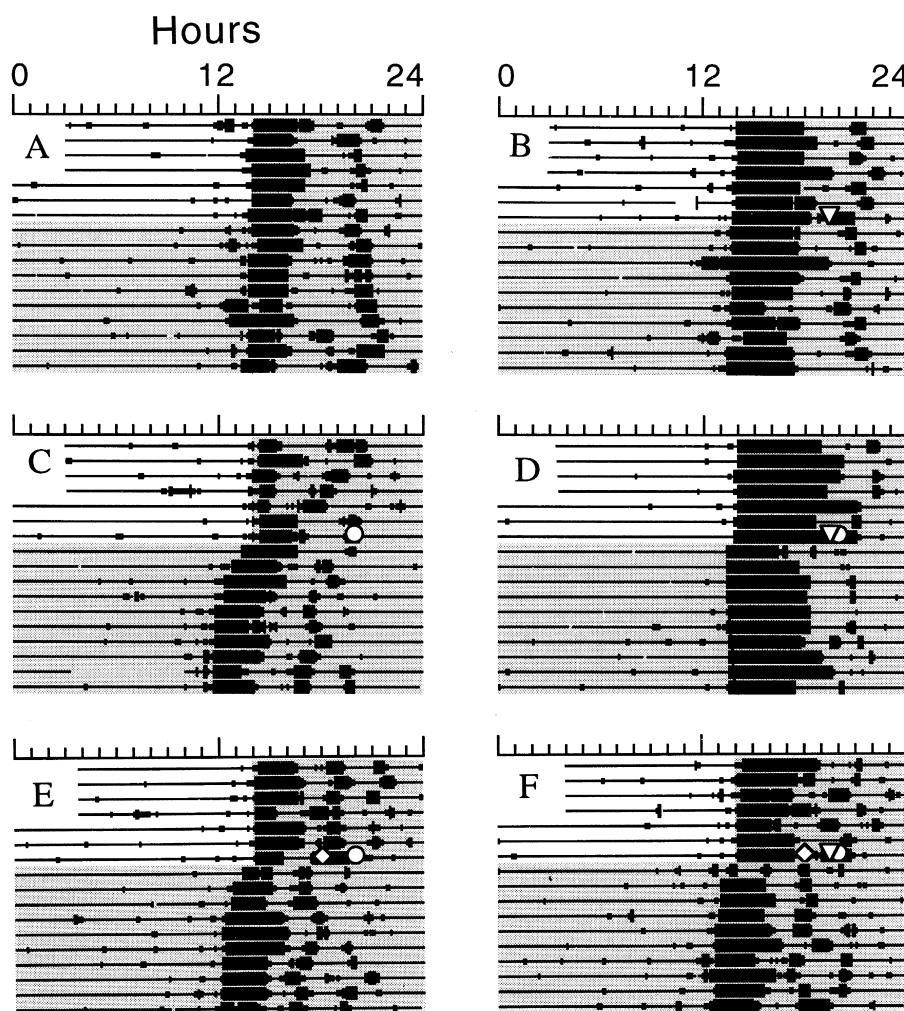


Fig. 2. Spontaneous home cage wheel running activity of a representative hamster in LD and DD (indicated by shading). Each line represents 24 h, in 10-min time bins plotted from left to right. Time bins during which activity counts were registered are indicated by bars proportional in height to the amount of activity (3 pts height = 1–9 revolutions (revs), 5 pts = 10–19 revs, 7 pts $\geq$ 19 revs). (A) Constant dark, no stimulus, (B) A bout of wheel running induced by confinement to a novel wheel for 45 min at ZT17.5 (denoted by arrowhead), (C) A light pulse at ZT18 (circle), (D) The combination of induced running and a light pulse, (E) Metergoline (diamond) followed by a light pulse, and (F) Metergoline followed by novel wheel confinement and a light pulse. In this example, the phase advance in response to light, measured on day 5 of DD, was 157 min, while in response to light combined with stimulated running was only 28 min.

computer algorithm for identifying the onset of the daily active period (the 10 min bin exceeding 50 wheel revolutions preceded by a 240 min interval in which counts did not exceed 50 in any bin). Light at ZT18 was expected to induce advance phase shifts stabilizing after several days of transient cycles [19]. Regression lines were therefore fit by computer to activity onsets for days 5–10 in DD, and this was extrapolated back to the day after the treatment. The phase shift was recorded as the difference in minutes between this intercept and that predicted on the basis of activity onsets during the last 5 days of LD. A second measure of phase shift was obtained by comparing the average activity onset during LD with activity onset on day 5 of DD. The advantage of using a single day 5 onset is that transient cycles were complete by this day, and a single day onset minimizes any contribution that a non-24 h free-running period (τ) makes to the phase shifts obtained using regression lines. Light exposure at ZT13 and 14 was expected to induce delay phase shifts with few or no transient cycles, therefore activity onset on day 3 of DD was used to quantify phase shifts, as described previously [27]. Phase shifts evident in the control condition (no treatments) were subtracted from phase shifts in response to light, activity and the combination, and the adjusted shifts were then compared by repeated measures ANOVA and two-tailed paired t -tests. Wheel revolutions during novel wheel confinement, light pulses and in the home cage were also quantified and compared by paired t -tests. Because activity was collected in 10 min intervals, the cumulative activity scores for wheel confinement and light pulses include the first 5 min following the end of the treatments.

3. Results

3.1. Phase shifts to light and activity at ZT18

Regression lines fit to days 5–10 of DD and activity onset on day 5 of DD produced very similar phase shift estimates. The slope of the regression lines yielded τ estimates that were slightly different from 24 h (range 23.97–24.05). However, there was no significant effect of treatment condition on τ ($F_{(4,60)} = 1.65$, $p > 0.1$) therefore τ is not likely to account for differences in phase shifts across treatments. Nonetheless, to minimize any contribution of τ , phase shift estimates obtained using day 5 activity onsets were used for statistical comparisons among treatments.

Mean phase shifts and running levels are summarized in Fig. 1a,b. Light pulses at ZT18 induced phase advance shifts of 81 ± 8 min ($t = 8.79$, $p < 0.001$, non-adjusted scores compared with DD alone; e.g., Fig. 2a,c). Wheel running induced by 45 min confinement to a novel wheel did not induce significant shifts by comparison to the control condition ($t = 0.28$, $p > 0.1$; Fig. 2b), but did

attenuate the phase shifting effect of light by $\sim 45\%$ (mean shift 43 ± 11 min; $t = 2.9$, $p < 0.01$ by comparison with light pulse alone; e.g., Fig. 2d).

Wheel running levels during novel wheel confinement were $\sim 400\%$ greater than spontaneous running levels in the home cage for that ZT during the control DD test (Fig. 1b). When novel wheel confinement was combined with a light pulse, activity levels were $\sim 300\%$ greater than in the light pulse alone condition ($t = 2.47$, $p < 0.02$).

3.2. Effects of metergoline on inhibition of light-induced shifts at ZT18 by activity

Pretreatment with metergoline did not significantly alter the phase shift in response to light (Fig. 2e), but did prevent the attenuation of light-induced shifting by novel

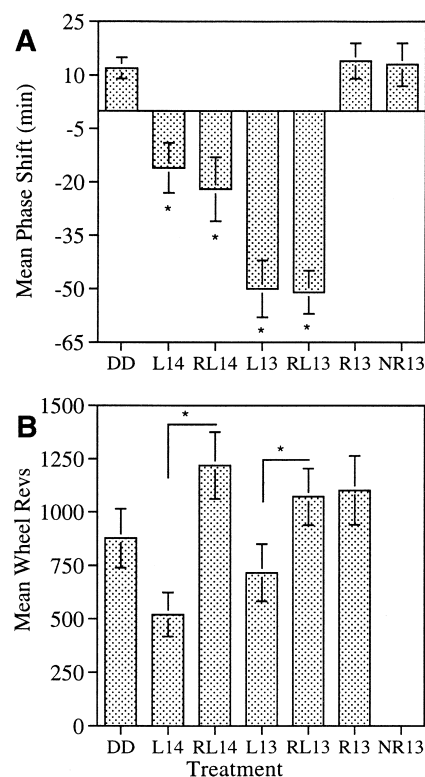


Fig. 3. (A) Group mean phase shifts $\pm$ S.E. in response to constant dark (DD), a 15-min light pulse at ZT14.25 (L14), the combination of 45 min of wheel running beginning at ZT13.75 and a light pulse at ZT14.25 (RL14), a light pulse at ZT13 (L13), the combination of 45 min of wheel running beginning at ZT12.5 and light at ZT13 (RL13), 45 min of wheel running beginning at ZT13.75 (R13), and 45 min with the running wheel locked (NR13). The mean for the DD condition represents the phase shift with respect to LD, while the means for the L and RL conditions represent the phase shifts adjusted by subtracting these DD shifts. The means for the R and NR conditions were from a separate group of hamsters, and represent shifts relative to LD. (B) Group mean wheel revolutions in 50 min at the ZT corresponding to novel wheel confinement. In panel A, * denotes significant difference from DD, R13 and NR13, and in panel B, * denotes significant difference between pairs ($p < 0.05$).

wheel running, i.e., following metergoline treatment, there was no significant difference between phase shifts in response to light alone and the combination of light and wheel running ($t = 0.04$, $p > 0.1$; e.g., Fig. 2f). Metergoline did not, on average, significantly suppress the running response to novel wheel confinement ($t = -0.92$, $p > 0.1$), indicating that inhibition of wheel running was not the mechanism by which metergoline prevented the inhibitory effects of novel wheel confinement on light-induced phase shifts. This interpretation was further evaluated by selecting 10 hamsters with the smallest difference in activity levels between the light pulse–novel wheel condition and the metergoline–light pulse–novel wheel condition (mean difference 40 ± 64 revolutions in 45 min). Although metergoline had virtually no effect on activity levels in this subsample, it nonetheless prevented inhibition of light-induced shifting by wheel running ($t = 1.08$, $p > 0.05$).

3.3. Phase shifts to light and activity at ZT13 and 14.25

Mean phase shifts and running levels are summarized in Fig. 3a,b. Light pulses at ZT14.25 induced small phase delays that were significant by day 3 of DD (-22 ± 9 min, $t = 3.81$, $p < 0.001$). Phase shifts to light at this ZT were not significantly altered by combining light pulses with confinement to a novel wheel ($t = 0.5$, $p > 0.1$),

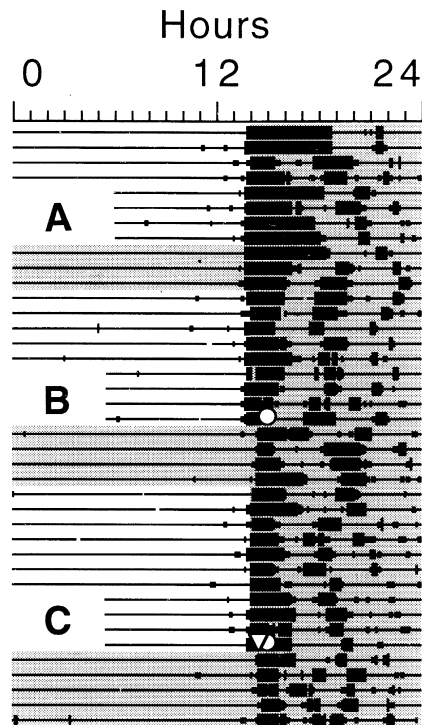


Fig. 4. Spontaneous home cage wheel running activity of a hamster in LD and DD. (A) Control DD test, no stimulus, (B) A light pulse at ZT13 (circle), (C) Wheel running induced by confinement to a novel wheel for 45 min at ZT12.5 (arrowhead) combined with a light pulse at ZT13. Other plotting conventions as in Fig. 2. Mean phase delay to light was 40 min, and to light combined with activity was 35 min.

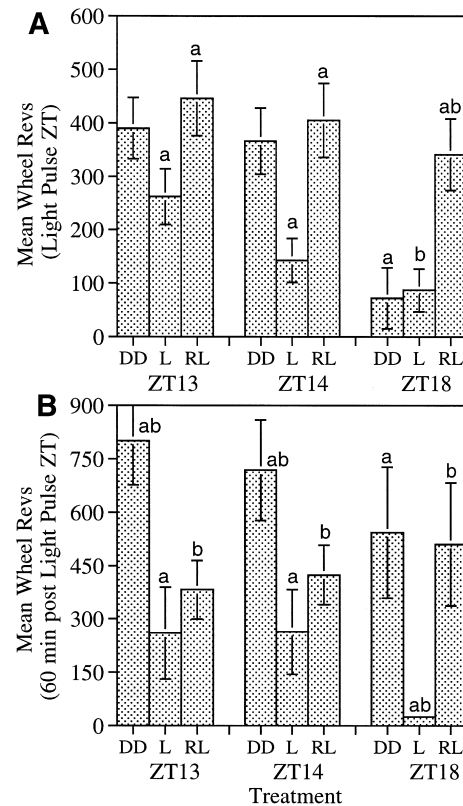


Fig. 5. (A) Group mean wheel revolutions $\pm$ S.E. for the 20 min period beginning at the ZT of light exposure in the light pulse (L) condition and the wheel running combined with light condition (RL), and at that ZT in the control condition (DD, no stimulus). (B) Group mean wheel revolutions $\pm$ S.E. for the 60 min period beginning 5 min after the end of light exposure in the L and RL conditions, and at that ZT in the control DD condition. Within each triad, letters denote means that are significantly different at $p < 0.05$.

although the novel wheel did induce a significant running response by comparison with the light-alone condition ($t = 4.28$, $p < 0.001$).

Light pulses at ZT13 induced larger phase delays (-50 ± 8 min; e.g., Fig. 4b) by comparison with light pulses at ZT14.25, and these were also not affected by running in a novel wheel ($t = 0.1$, $p > 0.1$; e.g., Fig. 4c), although running counts were significantly higher when hamsters were confined to novel wheels compared to the light pulse alone condition ($t = 2.61$, $p < 0.01$).

Running levels during wheel confinement did not differ significantly between the ZT13 and ZT14.25 light pulse conditions, and also did not differ significantly from home cage running levels at those ZTs on the DD control day. Home cage wheel running typically was at its highest levels early in the night in these hamsters, and this may represent a ceiling on wheel running that is not exceeded even by the stimulation of a novel wheel. Novelty-induced running levels during the ZT13 and ZT14.25 light pulses were greater than in the ZT18 condition, although this reached significance only for the ZT14.25–ZT18 comparison (independent $t = 2.45$, $p < 0.02$).

Neither confinement to novel wheels nor locking of home cage wheels for 45 min at ZT13.75 induced significant phase shifts by comparison with control DD tests (Fig. 3a).

3.4. Stability of entrained phase over time

Repeated measures ANOVA revealed no effect of time on the average phase of activity onset during LD prior to each DD test in group 1 (ZT18; $F_{(4,60)} = 1.11$, $p > 0.05$) and group 2 (ZT13.14; $F_{(4,70)} = 0.68$, $p > 0.05$) hamsters, indicating that there was no systematic change in the phase angle of entrainment as a consequence of repeated exposures to DD with and without light pulses and novel wheel confinement.

3.5. Extended inhibition of activity by light pulses

Activity levels were lower during the 15 min light pulses at ZT13 and ZT14.25, by comparison with the same ZTs during the DD control day, but variability was high and these differences did not reach the 0.05 level of significance (Fig. 5a). However, during the hour following the light pulses at both ZTs, activity was significantly attenuated by comparison with the same ZTs during the DD control day ($p < 0.01$ for both comparisons; Fig. 5b, Fig. 6a,b). This extended inhibition of activity following light pulses was attenuated when light was combined with novel wheel-induced running (Fig. 5b, Fig. 6c). The duration of quiescence following light pulses (defined as the time until at least 1 wheel revolution was recorded in each of 3 successive 10 min bins) was approximately 60–90 min, and tended to be shorter when light was paired with novelty-induced running, although this difference reached

significance only in the ZT14.25 comparison ($t = 3.51$, $p < 0.005$).

Light pulses at ZT18 did not significantly attenuate home cage wheel running, because activity levels were already quite low at that phase (Fig. 5a). However, many animals exhibited bimodal activity patterns, with a minor peak of activity beginning late in the night. Consequently, average home cage activity levels were increased after ZT18, although group variability was high (Fig. 5b). During the hour following the light pulse, activity levels were strongly suppressed by comparison with the control DD day ($t = 2.75$, $p < 0.02$). Confinement to a novel wheel during the light pulse prevented this effect (Fig. 5b).

4. Discussion

This study demonstrates that behavioral activity stimulated by confinement to a novel wheel can attenuate the phase advancing effect of a light pulse presented late in the subjective night in hamsters. These results therefore confirm one previous study that used a different design with potential confounds [32].

Stimulated running alone did not have a significant phase shifting effect late in the subjective night, therefore attenuation of light-induced phase advances was not due to induction of a competing phase delay. This finding is consistent with other studies showing that phase shifts to novelty-induced running in hamsters housed with free access to running wheels are consistently induced only when running exceeds 4000–5000 revolutions in a 3 h period, and that even at those high levels phase shifts to running late in the subjective night are small or absent [29].

A more likely explanation for attenuation of light-induced phase advances is that behavioral activity modulates transmission of photic information within the circadian system. Converging lines of evidence suggest that the mechanism by which behavior alters photic phase shifting may involve 5HT. The raphe nuclei are well positioned to modulate activity within the circadian system via direct projections from the median raphe to the SCN, and via projections from the dorsal raphe to the intergeniculate leaflet (IGL), which is a second major source of afferents to the SCN [22]. Numerous studies indicate that motor activity in several species is associated with increased neural activity in raphe nuclei and release of 5HT in terminal fields throughout the forebrain, including the SCN [8,18,37]. In addition, 5HT agonists can attenuate molecular and electrophysiological responses to light in the SCN [13,34,36] as well as behavioral phase shifts to light [14,34], and mixed 5HT1a antagonists can potentiate phase shifts to light [33]. Our observation that the general 5HT1 antagonist metergoline can prevent an inhibitory effect of behavior on light-induced phase shifting provides an important link between these previous findings, and supports a

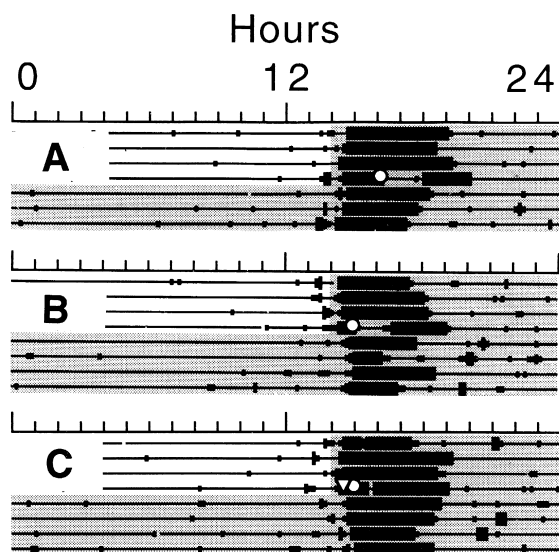


Fig. 6. Wheel running activity in a representative hamster exposed to light pulses at ZT14.25 (A), ZT13 (B), and ZT13 concurrent with a bout of novel wheel-induced running (C). Plotting conventions as in Fig. 2.

hypothesis that a major function of 5HT afferents within the circadian system is to mediate the effects of behavior or arousal on the response of the circadian pacemaker to light.

Although 5HT receptors in the SCN seem obvious candidates to mediate effects of activity-related 5HT release on photic shifts, an indirect effect via the IGL is also conceivable. Activation of serotonergic raphe units could result in autoinhibition via somatodendritic 5HT_{1a} autoreceptors. 5HT inhibits IGL neurons [43]. Attenuation of 5HT output by autoinhibition could thus disinhibit NPY containing cells of the IGL that project to the SCN. This could result in inhibition of SCN responses to light, as peri-SCN injections of NPY can block phase shifts to light *in vivo* [40], and NPY microdrops can block phase shifts to glutamate applied to SCN slices *in vitro* [4]. In addition, peri-SCN injections of anti-NPY have been shown to potentiate light-induced phase shifts *in vivo* [3]. Metergoline could prevent activation of NPY cells by blocking the somatodendritic 5HT_{1a} raphe autoreceptors. A critical test of this model requires measurement of 5HT levels in the IGL during behavioral activity late in the subjective night, and preliminary observations indicate that the model may have some validity (Glass, personal communication; see also Ref. [23]).

Consistent with known phase-response curves to light pulses in hamsters [19], brief light exposure early in the night induced significant phase delays of activity onset, and phase delays were greater to light at ZT13 than ZT14.25. Unlike phase advances to light late in the night, these phase delays were not affected by novel wheel-induced running. Neither running nor the absence of running early in the subjective night significantly altered circadian phase, so failure to observe attenuated photic shifting at this phase was not due to induction of a compensating phase delay. Also, novel wheel-induced running early in the night was marginally greater than that evident late in the night, so failure to observe an effect of activity early in the night was not due to low levels of stimulated running. Conceivably, behavioral activity might attenuate photic shifting early in the subjective night if the light pulse were dimmer or shorter, although we note that the light pulses used were below saturation, as we get larger shifts to light pulses of 300 lux at these ZTs (unpublished observations). The results, in any case, constitute evidence of circadian modulation of behavioral effects on light-induced phase resetting.

Failure of activity to modulate light-induced phase delays may be related to the high levels of home cage wheel running activity that are characteristically expressed during the first few hours of the night in hamsters entrained to LD 14:10 (e.g., Figs. 2, 4 and 6). Conceivably, 5HT receptors may be fully occupied or functionally downregulated at this time as a consequence of 5HT release associated with home cage running or other circadian factors, and these effects may persist through the 15 min light pulse, whether

or not the animals are induced to run during that time. The available evidence does indicate that extracellular 5HT levels in the SCN are maximal early in the night [37]. A ceiling effect on 5HT transmission may thus preclude detection of any modulating influence of behavior on light-induced phase shifts at this circadian phase. This model implies that light-induced phase shifts early in the subjective night may be larger if animals are sedentary or if 5HT activity is experimentally reduced (e.g., Ref. [33]).

In addition to its phase-shifting effects on activity rhythms, light can also acutely suppress activity in nocturnal rodents, thereby ‘masking’ the phase of the circadian pacemaker [2]. Suppression of activity by light was evident in the present study, although light did not prevent stimulation of activity by confinement to a novel wheel. An unexpected observation was that activity was significantly suppressed for 60–90 min after the light pulse was terminated. More detailed behavioral observations will be necessary to determine whether this period of quiescence is limited to wheel running or extends to other behaviors, including sleep. Evidence for such an effect has not been explicitly noted in the literature, possibly because the use of event records to represent activity data prior to the advent of microcomputers precluded analyses of wheel running levels. A possibly analogous finding was reported in a study of nocturnal flying squirrels housed in a semi-natural environment with a light-tight nest box; if at the end of their inactive phase the animals emerged from the nest while the lights were still on, they retreated to the nest and were quiescent for about 20 min, which approximated the size of the phase delay induced by the brief light exposure [7]. It remains to be determined whether the circadian system mediates, gates or plays any role in extended effects of light on behavior.

It may also be of interest that the period of quiescence evident following light exposure in the present study was attenuated when light was paired with novelty-induced running. This may be due to extended arousal induced by running, possibly involving compensatory eating, drinking or grooming behaviors, or it may be an indication that activity can attenuate an extended effect of light on behavioral state, as well as its effect on circadian phase.

Nonphotic stimuli can have surprisingly robust effects on the phase, period and form of circadian rhythms in the few species studied to date. The role of these effects in the behavioral ecology of any species is not yet well understood, and a major part of the task of unraveling their significance will be careful phase-response and dose-response analyses of nonphotic and photic stimuli in combination.

Acknowledgements

Supported by operating grants from NSERC to R.E.M. We thank Elliott Marchant for assistance with preliminary studies.

References

- [1] J. Aschoff, Response curves in circadian periodicity, in: J. Aschoff (Ed.), *Circadian Clocks*, North-Holland, Amsterdam, 1965, pp. 95–111.
- [2] J. Aschoff, S. Daan, K. Honma, Zeitgebers, entrainment and masking: some unsettled questions, in: J. Aschoff, S. Daan, G. Groos (Eds.), *Vertebrate Circadian Systems*, Springer-Verlag, New York, 1982, pp. 13–24.
- [3] S. Biello, Enhanced photic phase shifting after treatment with antiserum to neuropeptide Y, *Brain Res.* 673 (1995) 25–29.
- [4] S. Biello, D. Golombek, M. Harrington, Neuropeptide Y and glutamate block each other's phase shifts in the suprachiasmatic nucleus in vitro, *Neuroscience* 77 (1997) 1049–1058.
- [5] S. Biello, D. Janik, N. Mrosovsky, Neuropeptide Y and behaviorally induced phase shifts, *Neuroscience* 62 (1994) 273–279.
- [6] S. Biello, N. Mrosovsky, Blocking the phase-shifting effect of neuropeptide Y, *Proc. R. Soc. London, Ser. B* 259 (1995) 179–187.
- [7] P. DeCoursey, Light-sampling behavior in photoentrainment of a rodent circadian rhythm, *J. Comp. Physiol. A* 159 (1986) 161–169.
- [8] T. Dudley, J. Glass, Endogenous 5-HT release in the Syrian hamster SCN, *Soc. Res. Biol. Rhythms Abst.* 5 (1996) 51.
- [9] F. Ebling, The role of glutamate in the photic regulation of the suprachiasmatic nucleus, *Prog. Neurobiol.* 50 (1996) 109–132.
- [10] D. Edgar, W. Dement, Regularly scheduled voluntary exercise synchronizes the mouse circadian clock, *Am. J. Physiol.* 261 (1992) R928–R933.
- [11] D. Edgar, M.R. Dean, W. Dement, Serotonergic afferents mediate activity-dependent entrainment of the mouse circadian clock, *Am. J. Physiol.*, in press (1997).
- [12] D. Edgar, C. Martin, W. Dement, Activity feedback to the mammalian circadian pacemaker: influence on observed measures of rhythm period length, *J. Biol. Rhythms* 6 (1991) 185–199.
- [13] J. Glass, M. Selim, M. Rea, Modulation of light-induced *c-fos* expression in the suprachiasmatic nuclei by 5-HT_{1A} receptor agonists, *Brain Res.* 638 (1994) 235–242.
- [14] J. Glass, M. Selim, G. Srkalovic, M. Rea, Tryptophan loading modulates light-induced responses in the mammalian circadian system, *J. Biol. Rhythms* 10 (1995) 80–90.
- [15] W. Hohmann, S. Michel, G. Fleisner, Locomotor activity and light pulses as competing zeitgeber stimuli in the scorpion circadian system, *Soc. Res. Biol. Rhythms Abst.* 2 (1990) 52.
- [16] G. Honrado, N. Mrosovsky, Arousal by sexual stimuli accelerates the re-entrainment of hamsters to phase-advanced light–dark cycles, *Behav. Ecol. Sociobiol.* 25 (1989) 57–63.
- [17] G. Honrado, N. Mrosovsky, Interaction between periodic socio-sexual cues and light–dark cycles in controlling the phasing of activity rhythms in golden hamsters, *Ethol. Ecol. Evol.* 3 (1992) 221–231.
- [18] B. Jacobs, E. Azmitia, Structure and function of the brain serotonin system, *Physiol. Rev.* 72 (1992) 165–229.
- [19] C. Johnson, *An Atlas of Phase Response Curves for Circadian and Circatidal Rhythms*, Department of Biology, Vanderbilt Univ., Nashville, TN, 1990.
- [20] E. Marchant, R. Mistlberger, Entrainment and phase shifting of circadian rhythms in mice by forced treadmill running, *Physiol. Behav.* 60 (1996) 657–663.
- [21] E.G. Marchant, N.V. Watson, R.E. Mistlberger, Both neuropeptide Y and serotonin are necessary for entrainment of circadian rhythms in mice by daily treadmill running schedules, *J. Neurosci.* 17 (1997) in press.
- [22] E. Meyer-Bernstein, L. Morin, Differential serotonergic innervation of the suprachiasmatic nucleus and the intergeniculate leaflet and its role in circadian rhythm modulation, *J. Neurosci.* 16 (1996) 2097–2111.
- [23] E. Mintz, C. Gillespie, C. Marvel, K. Huhman, H. Albers, Serotonergic regulation of circadian rhythms in Syrian hamsters, *Neuroscience* 79 (1997) 563–569.
- [24] R. Mistlberger, Effects of daily schedules of forced activity on free-running rhythms in the rat, *J. Biol. Rhythms* 6 (1991) 71–80.
- [25] R. Mistlberger, Circadian food-anticipatory activity: Formal models and physiological mechanisms, *Neurosci. Biobehav. Rev.* 18 (1994) 171–195.
- [26] R. Mistlberger, B. Rusak, Circadian rhythms in mammals: formal properties and environmental influences, in: M. Kryger, T. Roth, W. Dement (Eds.), *Principles and Practice of Sleep Medicine*, Saunders, Philadelphia, PA, 1994, pp. 277–288.
- [27] R. Mistlberger, S. Sinclair, E. Marchant, L. Neil, Circadian phase shifts to food deprivation and refeeding in the Syrian hamster are mediated by running activity, *Physiol. Behav.* 61 (1996) 273–278.
- [28] N. Mrosovsky, Double pulse experiments with nonphotic and photic phase-shifting stimuli, *J. Biol. Rhythms* 6 (1991) 167–179.
- [29] N. Mrosovsky, Locomotor activity and non-photic influences on circadian clocks, *Biol. Rev.* 71 (1996) 343–372.
- [30] N. Mrosovsky, P. Salmon, A behavioural method for accelerating re-entrainment of rhythms to new light–dark cycles, *Nature* 330 (1987) 372–373.
- [31] P.D. Penev, P.C. Zee, F.W. Turek, Serotonin in the spotlight, *Nature* 385 (1997) 123.
- [32] M. Ralph, N. Mrosovsky, Behavioral inhibition of circadian responses to light, *J. Biol. Rhythms* 7 (1992) 353–360.
- [33] M. Rea, J. Barrera, J. Glass, R. Gannon, Serotonergic potentiation of photic phase-shifts of the circadian activity rhythm, *NeuroReport* 6 (1995) 1289–1292.
- [34] M. Rea, J. Glass, C. Colwell, Serotonin modulates photic responses in the hamster suprachiasmatic nuclei, *J. Neurosci.* 14 (1994) 3635–3642.
- [35] S. Reeb, N. Mrosovsky, Effects of induced wheel running on the circadian activity rhythms of Syrian hamsters: entrainment and phase-response curve, *J. Biol. Rhythms* 4 (1989) 39–48.
- [36] M. Selim, J. Glass, U. Hauser, M. Rea, Serotonergic inhibition of light-induced Fos protein expression and extracellular glutamate in the suprachiasmatic nuclei, *Brain Res.* 621 (1993) 181–188.
- [37] T. Shioiri, K. Takahashi, N. Yamada, S. Takahashi, Motor activity correlates negatively with free-running period, while positively with serotonin contents in SCN in free-running rats, *Physiol. Behav.* 49 (1991) 779–786.
- [38] S.V. Sinclair, R.E. Mistlberger, Activity reorganizes circadian phase of Syrian hamsters under full and skeleton photoperiods, *Behav. Brain Res.* (1997) in press.
- [39] F. Turek, Effects of stimulated physical activity on the circadian pacemaker of vertebrates, *J. Biol. Rhythms* 4 (1989) 135–149.
- [40] E.T. Weber, M. Cagle, M.A. Rea, Local neuropeptide Y blocks light-induced phase advances of the circadian wheel-running activity rhythm in the syrian hamster, *Soc. Neurosci. Abst.* 21 (1995) 454.
- [41] D. Welsh, G. Richardson, W. Dement, Effect of running wheel availability on circadian patterns of sleep and wakefulness in mice, *Physiol. Behav.* 43 (1988) 771–777.
- [42] N. Yamada, K. Shimoda, S. Ohi, S. Takahashi, K. Takahashi, Free-access to a running wheel shortens the period of free-running rhythms in blinded rats, *Physiol. Behav.* 42 (1988) 87–91.
- [43] S. Ying, D. Zhang, B. Rusak, Effects of serotonin agonists and melatonin on photic responses of hamster intergeniculate leaflet neurons, *Brain Res.* 628 (1993) 8–16.