

Sleep deprivation can attenuate light-induced phase shifts of circadian rhythms in hamsters

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Abstract

To determine whether sleep deprivation (SD) affects the response of circadian rhythms to light, hamsters were forced to walk on a slowly rotating treadmill for 6 or 24 h, ending early in the night, with or without a light pulse during the last 30 min. SD alone did not produce a significant phase shift. Light pulses (300 and 50 lx) alone induced significant delay shifts (55 and 35 min, respectively). Twenty-four hours SD significantly attenuated the delay to brighter light and 6 h SD significantly attenuated the delay to moderate light. Sleep loss or attendant low-intensity continuous activity appear to modulate the response of the hamster circadian system to light. © 1997 Elsevier Science Ireland Ltd.

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Shift work rotation and jet travel are often accompanied by acute or chronic sleep loss, but little work has been done to determine whether this might affect the rate at which circadian rhythms adapt to new time schedules. Sleep deprivation (SD) could affect reentrainment rates in at least two ways; it could induce phase shifts synergistic or antagonist to the direction of reentrainment, or it could potentiate or attenuate the circadian pacemaker's phase shift response to light. The few animal studies available have produced mixed results; one study found that 24 h SD (continuous activity in a rotating drum) did not alter the phase or period of sleep-wake circadian rhythms in rats free-running in constant dark (DD) [3], whereas a second study reported that 24 h SD (platform method) was associated with phase shifts, splitting or general disruptions of activity rhythms in hamsters free-running in constant light [18]. No study has yet examined phase shift responses to light pulses during or after forced SD in an animal model. Herein we report studies of sleep deprivation and circadian phase resetting to light in the Syrian hamster. This species was chosen because

it exhibits sleep rebounds and physiological responses to SDs as short as 4 h [2,19].

Male Syrian hamsters (Charles River, Montreal, Canada) were housed individually at 3 months old in plastic cages with 17 cm running wheels monitored by computer. Activity counts were summed and stored at 10 min intervals and periodically downloaded to a Macintosh computer for display and analyses. SDs were accomplished by transporting hamsters to an eight-lane treadmill, rotating at 2.4 m/min. Slow movement was enforced by occasional prodding and compressed air puffs. Food pellets and water were available in the treadmill and were consumed. Light pulses, with or without SD, were given in the moving treadmill. Phase shifts were assessed using the Aschoff Type II procedure [1,13], i.e. the light pulse was applied on the first night of DD and activity onset time on subsequent days of DD was compared to the average onset time during the last 3–4 days under the prior light-dark (LD) cycle. Animals were first recorded in LD 14:10 (20:1 lx white/red), then in DD (1 lx red) for 3–5 days, beginning at the usual time of dark onset. Each animal was subjected to at least four DD tests, separated by 10 days of LD, to assess phase shift responses to light, SD, and the two combined. Day 3 of DD was used to calculate phase shifts (previous studies and preliminary

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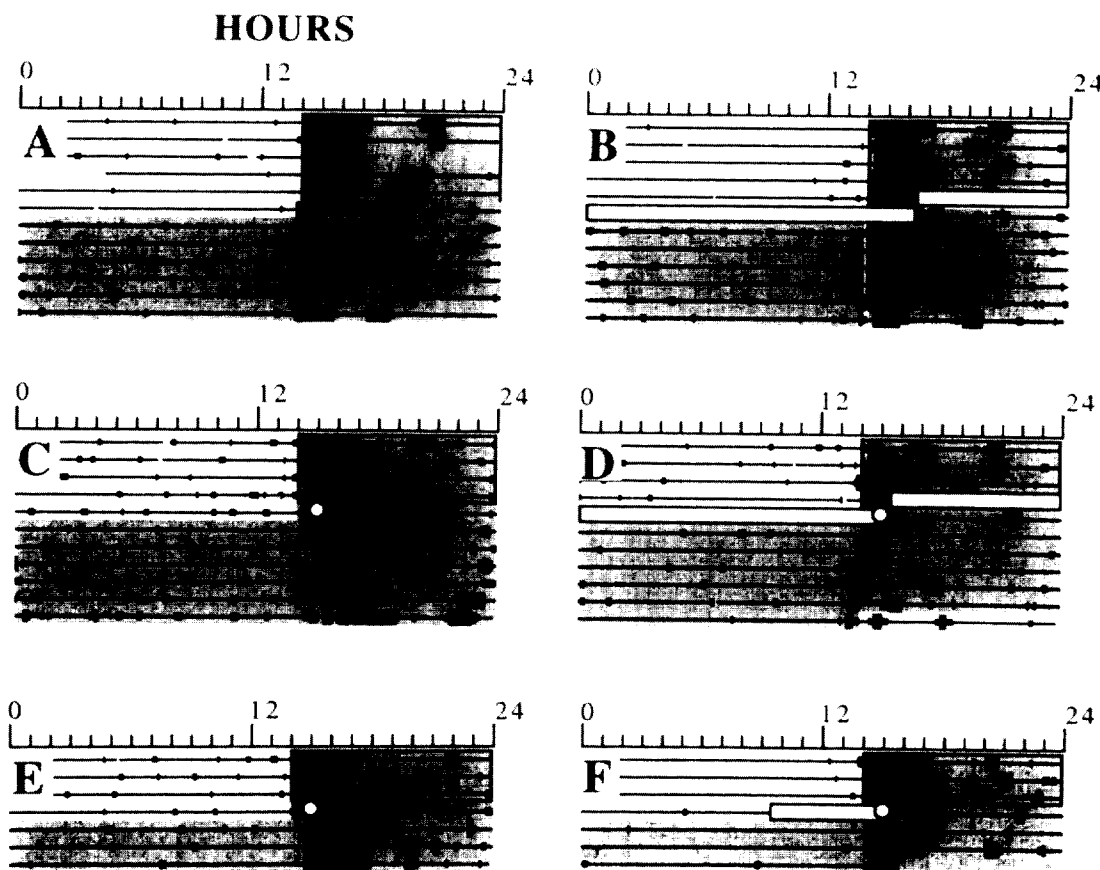


Fig. 1. Spontaneous, home cage wheel running activity of representative hamsters subjected to light pulses and sleep deprivation. Each line represents a day, plotted in 10 min bins from left-to-right. Time bins during which wheel running occurred are indicated by dark bars of varying heights (three points, 1–9 revs; five points, 10–19 revs; seven points, 20 revs). Lights-out (dim red ~1 lx) is represented by stippling. Sleep deprivation is indicated by open bars, and light pulses by open circles. (A) Control condition; no stimulus, minimal shift of activity onset. (B) 24 h SD, minimal shift. (C) Thirty minutes, 300 lx light pulse, a 50 min phase delay by the third cycle after the light pulse. (D) Twenty-four hours SD and 30 min, 300 lx light pulse, a 20 min phase advance by the third cycle. (E) Thirty minutes, 50 lx light pulse, a 40 min phase delay. (F) Six hours SD and 30 min, 50 lx light pulse, a 10 min phase delay.

analysis indicated that phase delay shifts to light are fully expressed by that day; for extensive review, see [10]). A control DD test with neither light nor SD served to establish the amount of shift attributable to transfer into DD. Mean shifts (reported $\pm$ SE) were compared by repeated measures analysis of variance, with paired post-hoc *t*-tests.

One group of hamsters ($n = 8$) was first subjected to a DD control test, a 24 h SD, a 30 min/300 lx light pulse, and SD and light combined. SDs began at 'zeitgeber time' (ZT) 14.5 on the last night of LD (i.e. 2.5 h after dark onset, which is designated ZT12 by convention) and ended at ZT14.5 on the first night of DD. The light pulse began at ZT14 and overlapped with the last 30 min of SD. A single light pulse at this time would be expected to produce a phase delay of activity onset on subsequent days [10]. On the first 3 days of DD in the control condition (no SD or light), the onset of spontaneous home cage wheel running activity was shifted -1 ± 3 min ($-$ denotes a delay), 9 ± 3 min, and 9 ± 3 min, respectively, by comparison with the average activity onset time during the last 3 days of LD (Figs. 1A and 2). Twenty-four hours SD ending at ZT14.5 did not produce a phase shift

significantly different from the control condition (DD day 3 mean = 7 ± 4 min; Fig. 1B and Fig. 2). Light pulses at ZT14 resulted in only a trend toward the expected phase delay shift (-8 ± 11 min, $P > 0.05$). Twenty-four hours SD prior to light blocked this trend (4 ± 5 min, $P > 0.05$).

This first set of conditions demonstrated that using the Aschoff Type II procedure for assessing phase shifts, 24 SD by slow treadmill running does not significantly perturb circadian phase. There was also a suggestion that SD might prevent phase delay shifts to light, but the phase shifts to the light pulses alone were, in aggregate, quite small. To further evaluate effects of SD on light-induced shifting, the hamsters were subjected to 30 min/300 lx light pulses 1 h earlier, at ZT13, with and without 24 h SD. Also, to evaluate the effect of treadmill running during the light pulses, hamsters received a second 30 min light pulse, without prior SD, in which the treadmill was stationary. Light pulses with the treadmill moving induced 57 ± 4 min immediate phase delays (i.e. no transient cycles evident; $F_{(3,27)} = 26.2$, $P < 0.001$; e.g. Figs. 1C and 2), which did not differ significantly ($P > 0.05$) from the 45 ± 8 min phase delays to light when

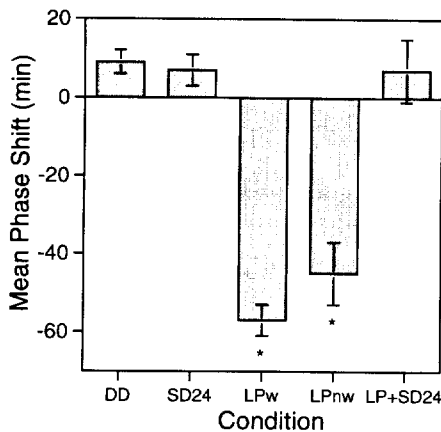


Fig. 2. Group mean ($\pm$ SE) phase shifts in the control condition (DD, no stimulus), after 24 h SD (SD24), after a 30 min, 300 lx light pulse at ZT13, with the treadmill rotating (LPw), after a 30 min, 300 lx light pulse at ZT13, with the treadmill stationary (LPnw), and after a 30 min, 300 lx light pulse combined with 24 h SD.

the treadmill was stationary (Fig. 2). Phase shifts to light pulses were significantly attenuated by 24 h SD (7 ± 10 min advance, $P < 0.001$; Figs. 1D and 2). These results demonstrate that phase shifts to light are not affected by slow running (2.4 m/min) during a light pulse, but are significantly attenuated by 24 h SD.

During the latter part of the 24 h SD, hamsters attempted to sleep by riding the treadmill, and required increasing levels of stimulation to maintain movement. In a second group of hamsters ($n = 8$) we evaluated whether a less rigorously enforced SD (24 h, with more passive treadmill riding permitted), or a shorter SD (6 h, beginning at ZT7, with SD carefully enforced) can also attenuate phase delays to light at ZT13. In view of the shorter duration of SD, a light pulse of moderate (30 min, 50 lx), rather than high intensity was used. These light pulses produced smaller but significant delay shifts (-36 ± 6 min; $F_{(3,27)} = 13.5$, $P < 0.001$; Figs. 1E and 3). The 24 h SD with reduced stimulation, and therefore increased passive riding, did not significantly attenuate light-induced phase delays (-28 ± 7 min, $P > 0.05$). However, the 6 h SD did produce significant attenuation (-5 ± 3 min, $P < 0.01$; Figs. 1F and 3).

These results show that in hamsters, as little as 6 h of SD during the last day of LD, ending during the first night of DD, can significantly attenuate phase shift responses to light pulses of moderate to high intensity presented early in the animals' night (active period). SD alone did not produce significant phase shifts, so attenuation was not due to induction of competing phase advance shifts. Although the hamsters were active during the SD, previous studies have shown that activity must be of high intensity to induce significant phase shifts [8]. One previous study reported that 24 h SD shifted or disrupted activity rhythms in hamsters, but that may be dependent on the use of constant light, which promotes rhythm damping and splitting in rodents [18].

The mechanism by which SD affects sensitivity of the

circadian pacemaker to photic stimulation remains to be elucidated. However, several recent lines of work suggest that it may involve increased 5HT release in the hypothalamic suprachiasmatic nucleus (SCN), the master circadian pacemaker that mediates photic entrainment [11]. First, there is evidence that 5HT inhibits photic sensitivity of the SCN pacemaker; this has been established at the behavioral [15,16], electrophysiological [12,16,20] and molecular levels [6,17]. Second, there is evidence that short-term SD can increase 5HT activity in the hypothalamus [2]. Finally, recent studies indicate that sleep deprivation desensitizes 5HT_{1a} inhibitory autoreceptors in the raphe nuclei, which would be expected to enhance release of 5HT in raphe projection sites, including the SCN [14]. A prediction from this 5HT hypothesis would be that neurotoxic lesions of 5HT terminals in the SCN should prevent attenuation of light-induced phase shifts by SD.

Whether locomotor activity plays a central role in the effect of SD on phase shifts to light is unclear. Treadmill rotation used in this study was very slow, and did not affect phase shifts to light in non-deprived animals. In addition, hamsters placed in the treadmill for 24 h, but subjected to less stimulation, thereby permitting more passive ride time and microsleeps, did not show attenuation of light-induced phase shifts, although they did have to move approximately the same distance in 24 h as in the other 24 h SD conditions. However, locomotor activity is associated with increased activity of raphe nuclei and release of 5HT [5,7], so it is conceivable that even low intensity activity, if prolonged, might enhance 5HT transmission in the SCN, either by itself or via an interaction with other neural consequences of sleep loss. Stress due to sleep loss is unlikely to play a role, as we have found no effect of stress (30 min continuous exposure to compressed air) during a light pulse on phase shifting (unpublished observations).

The effects of sleep deprivation and activity on the

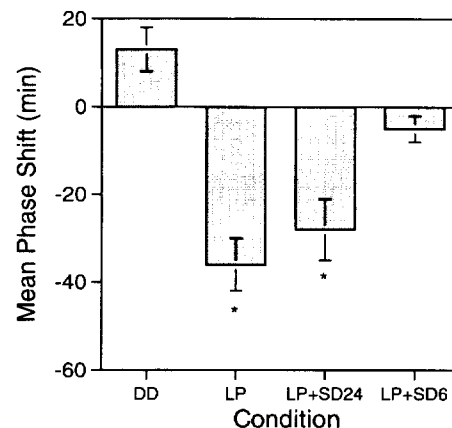


Fig. 3. Group mean ($\pm$ SE) phase shifts in the control condition (DD, no stimulus), after a 30 min, 50 lx light pulse at ZT13, with the treadmill rotating (LP), after a 30 min, 50 lx light pulse at ZT13 combined with 24 h SD (LP + SD24), and after a 30 min, 50 lx light pulse combined with 6 h SD (LP + SD6).

response of the circadian system to light pulses have not yet been systematically studied in humans. Bright light pulses can shift circadian phase in sleep deprived humans that are supine (i.e. completely sedentary) throughout the procedure [4]. The results presented here suggest that phase-response and dose-response studies of sleep deprivation, activity and light in humans would be of interest.

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