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POTENT CIRCADIAN EFFECTS OF DIM ILLUMINATION AT NIGHT IN HAMSTERS

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Conventional wisdom holds that the circadian pacemaker of rodents and humans is minimally responsive to light of the intensity provided by dim moonlight and starlight. However, dim illumination (<0.005 lux) provided during the daily dark periods markedly alters entrainment in hamsters. Under dimly lit scotophases, compared to completely dark ones phases, the upper range of entrainment is increased by ~4 h, and re-entrainment is accelerated following transfer from long to short day lengths. Moreover, the incidence of bimodal entrainment to 24 h light:dark:light:dark cycles is increased fourfold. Notably, the nocturnal illumination inducing these pronounced effects is equivalent in photic energy to that of a 2 sec, 100 lux light pulse. These effects may be parsimoniously interpreted as an action of dim light on the phase relations between multiple oscillators comprising the circadian pacemaker. An action of dim light distinct from that underlying bright-light phase-resetting may promote more effective entrainment. Together, the present results refute the view that scotopic illumination is environmental "noise" and indicate that clock function is conspicuously altered by nighttime illumination like that experienced under dim moonlight and starlight. We interpret our results as evidence for a novel action of dim light on the coupling of multiple circadian oscillators.

Keywords Circadian rhythms, Splitting, Coupling, Entrainment, Oscillator interactions, Light intensity effects, Hamsters, Dim light

INTRODUCTION

In most mammalian species studied, light is the principal environmental cue that influences endogenous circadian pacemakers. Two hallmark circadian responses to light—phase-shifting and melatonin suppression—exhibit a monotonic dependence on light intensity described by a sigmoidal function in a log-linear plot (Brainard et al., 1982, 1984; Nelson and Takahashi, 1991a, 1991b; Podolin et al., 1987;

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Takahashi et al., 1984). While absolute thresholds are difficult to define precisely, robust phase-shifting is not generally evident in the Syrian hamster with 5 min of 503 nm monochromatic light $<10^{11}$ photons $cm^{-2} sec^{-1}$ or <0.1 lux (Nelson and Takahashi, 1991a). Light-induced suppression of melatonin secretion is slightly more sensitive (Nelson and Takahashi, 1991b). As circadian thresholds approximate the luminance from a full moon, it has been hypothesized that these thresholds represent adaptations to prevent interference with stable entrainment (Brainard et al., 1984; Nelson and Takahashi, 1991a). This hypothesis is supported by the finding that deer mice that inhabit woodlands, where moonlight is greatly attenuated by the tree canopy, exhibit thresholds an order of magnitude lower than do hamsters that dwell in a desert landscape (DeCoursey, 1990).

If circadian visual systems are indeed blind to naturally occurring ambient levels of dim nighttime illumination, then it should be adequate to simulate nighttime in the lab with either complete darkness or dim red light, as commonly practiced. On the other hand, the absence of robust phase-shifts or melatonin suppression in response to dim moon-light intensities does not preclude other functionally significant effects of nighttime illumination on circadian systems. Supporting this latter possibility, we demonstrated potent effects of dim illumination versus complete darkness at night in several circadian entrainment paradigms.

METHODS

General Matters

Female and male Siberian and Syrian hamsters were studied. Animal housing, care, and application of experimental procedures were consistent with the standards of the Journal (Touitou et al., 2004). In each of the several entrainment paradigms described below, hamsters were exposed to lighting regimes alternating between bright white light (~100 to 300 lux) generated by white fluorescent bulbs (photophase conditions) and relative darkness (scotophase conditions). In each study, the scotophase condition was either complete darkness or dim nocturnal illumination provided by green light-emitting diodes with a peak bandwidth at 560 nm and a half-bandwidth of 23 nm. The specific configurations of the lamps differed between experiments, but illumination within the brightest region of the cage typically did not exceed ~0.005 lux. Dim-light irradiance was measured at 7.9×10^{-6} μW cm^{-2}, which is equivalent to 2.23×10^{9} photons cm^{-2} sec^{-1}.

Entrainment Paradigms

T-Cycle Entrainment

Male Syrian hamsters were transferred from light-dark (LD) 14 : 10 to T-cycles that lengthened abruptly to 25 h and then progressively to 30 h via 20 min increments at weekly intervals ($n = 18$/group). The original 14 h photophase was replaced by two 4.5 h "skeleton" pulses throughout the experiment (Figure 1A and 1B). General locomotor activity was monitored by passive infrared motion detectors placed above the cage (Gorman et al., 2005).

24 h LDLD Cycles

Male and female Syrian and male Siberian hamsters ($n = 7$ to 13/group) were transferred from group housing in LD 14 : 10 without running wheels to individual, wheel-running cages in LDLD 7 : 5 : 7 : 5 (Figure 1C and 1D) (Gorman et al., 2003; Gorman and Elliott, 2004; Evans et al., 2005).

Short-Day Re-entrainment

Male Siberian hamsters were transferred from group housing in LD 19 : 5 to single housing in LD 10 : 14 without running wheels (Figure 1E and 1F; $n = 17$ to 18/group). Activity was monitored by motion detectors in the absence of wheels (Gorman and Elliott, 2004).

Skeleton Photoperiods

Female Syrian hamsters were transferred from LD 14 : 10 to wheel-running cages in LD 17 : 7 and subsequently to a skeleton photoperiod of LD 17 : 7 with 3 h light pulses (see Figure 1G and 1H; $n = 16$/group). At weekly intervals thereafter, the skeleton photoperiod was altered to reduce the length of the original nighttime scotophase in 30 min increments (Evans et al., 2005).

RESULTS

Figure 1 illustrates representative entrainment patterns under each of the entrainment paradigms described above. Figure 1A shows a hamster exposed to completely dark nights that fails to entrain to T-cycles longer than 26 h but instead free-runs with a period near 24 h. The plotting of the lengthening T-cycle in 360 angular degrees results in the appearance of an accelerating free-running rhythm, although it remains near 24 h in this plot. In contrast, the representative hamster exposed to the same

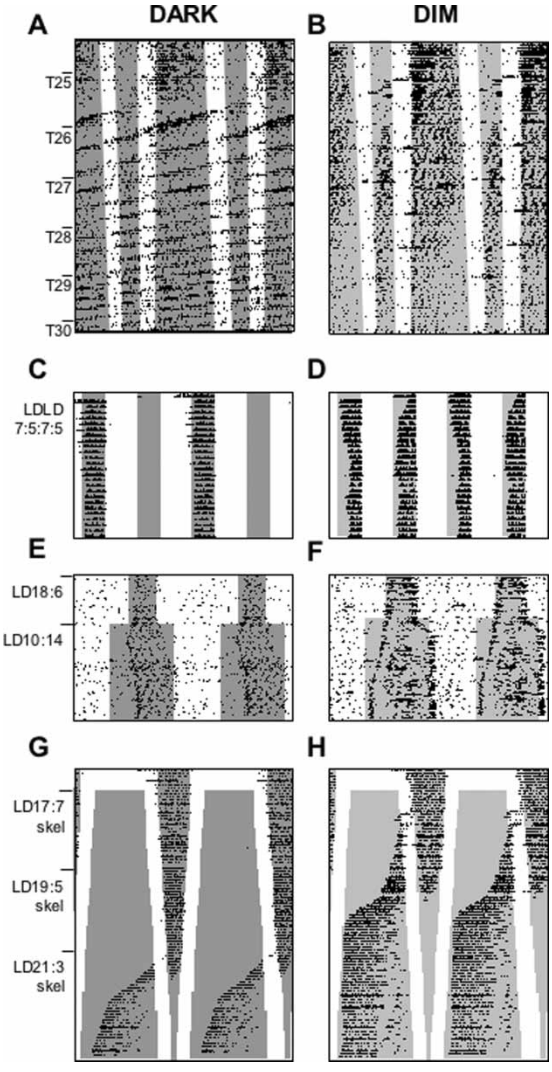

FIGURE 1 Activity records of Syrian and Siberian hamsters exposed to entrainment regimens with complete darkness (*left panels*) or dim illumination (*right panels*) during each scotophase. **Panels A and B:** Representative records of male Syrian hamsters under non 24 h T cycles. Actograms are double-plotted modulo-T with abscissa scaled in 360 angular degrees. Loss of entrainment is indicated by a break from the vertical alignment of activity onsets. Nearly all hamsters given darkness break entrainment before T = 26 h, whereas most animals under dim conditions entrain to T = 28 h or longer (Gorman et al., 2005). **Panels C and D:** Wheel-running records of male Syrian hamsters maintained in 24 h light : dark : light : dark (LDLD) cycles. In hamsters, the unimodal rhythm predominates under dark nights, whereas split rhythms are induced under dim illumination (Evans et al., 2005; Gorman and Elliott, 2004; Gorman et al., 2003). **Panels E and F:** Actograms of male Siberian hamsters transferred from LD 19 : 5 to LD 10 : 14. Under dark nights, hamsters expand α slowly or not at all. Dim light accelerates the lengthening of α, promotes testis regression, and prevents short-day nonresponsiveness (Gorman and Elliott, 2004). **Panels G and H:** Wheel-running records of Syrian hamsters under progressively lengthening skeleton photoperiods. Relative to dark nights, dimly lit nights decreased the latency to initiate a phase jump. Once initiated, the phase-jump occurred more gradually in dark versus dim light conditions (Evans et al., 2005).

bright-light cycle paired with dim nocturnal illumination (Figure 1B) remains entrained through T = 30 h. Periodogram analyses support this characterization (Gorman et al., 2005).

Under 24 h LDLD cycles (Figure 1C), hamsters exposed to dark scotophases typically entrain with a single nocturnal activity phase that anticipates one of the two daily scotophases (*e.g.*, 11 of 13 hamsters in Gorman et al., 2003). In contrast, a majority of hamsters exposed to dim nighttime illumination entrains by dividing their activity into two components—one associated with each of the daily scotophases (12 of 13 animals).

Following transfer from long (LD 19 : 5) to short (LD 10 : 14) photoperiods, hamsters housed under dark nights gradually lengthen their active phase (Figure 1E). A substantial fraction of hamsters termed "nonresponders," however, retains a long photoperiod activity pattern and thus fails to undergo gonadal regression (not shown). With the addition of dim nighttime illumination, activity duration lengthens more rapidly (Figure 1F), and hamsters are uniformly photoresponsive.

Under skeleton photoperiods (Figure 1G), hamsters with dark nights remain well entrained as the original night is progressively shortened. Only when the night is reduced to ~3.5 h do hamsters with dark scotophases exhibit a phase jump, where activity re-entrains into the second longer scotophase previously associated with subjective day. In contrast, under dimly lit nights (Figure 1H), phase jumps occur substantially earlier, when the original night is ~5 h in length.

DISCUSSION

Far from being biologically inefficacious, very dim illumination at night is a potent modulator of circadian function. The dim light used in these studies is of an intensity likely to be experienced by hamsters in nature. Thus, the use of completely dark scotopic conditions in the laboratory may be a poor simulation of relevant natural conditions. Additionally, given the ease of delivery of nighttime illumination and the size of its effects, dim light may prove to be a valuable tool for the therapeutic manipulation of human circadian function.

In each of the paradigms used in the studies described here, the system is entrained or re-entrained in the presence of bright light (~100 to 300 lux), raising the possibility that dim light potentiates the effects of bright light. While there may be instances in which animals phase-shift more readily under dim than under dark background conditions (Evans et al., 2005), well controlled studies show that the bright light pulse PRC is very similar under dimly lit *versus* dark background conditions (J.A. Evans, J.A. Elliott, and M.R. Gorman, unpublished results). Instead, the intrinsic state of the pacemaker is altered by this dim illumination, as reflected in a markedly lengthened active phase (α) relative to that

displayed in darkness. This result mirrors the longer α's reported in hamsters under T-cycles and short day photoperiods, and this change would be expected to alter entrainment to bright-light regimes (Pittendrigh et al., 1984; Elliott and Kripke, 1998).

Each of the entrainment paradigms used in this study is best understood in terms of a multi-oscillator pacemaker with flexible phase relationships between the component oscillators. These phase relationships are influenced not only by bright light but by nonspecified interactions between oscillators (*e.g.*, coupling). The convergent effects of dim light within each of the present paradigms suggest a possible site of action on oscillator coupling and may present an opportunity to investigate this fundamental property of circadian organization in a new manner.

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