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Time-Dependent Effects of Dim Light at Night on Re-Entrainment and Masking of Hamster Activity Rhythms

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Abstract Bright light has been established as the most ubiquitous environmental cue that entrains circadian timing systems under natural conditions. Light equivalent in intensity to moonlight (<1 lux), however, also strongly modulates circadian function in a number of entrainment paradigms. For example, compared to completely dark nights, dim nighttime illumination accelerated re-entrainment of hamster activity rhythms to 4-hour phase advances and delays of an otherwise standard laboratory photocycle. The purpose of this study was to determine if a sensitive period existed in the night during which dim illumination had a robust influence on speed of re-entrainment. Male Siberian hamsters were either exposed to dim light throughout the night, for half of the night, or not at all. Compared to dark nights, dim illumination throughout the entire night decreased by 29% the time for the midpoint of the active phase to re-entrain to a 4-hour phase advance and by 26% for a 4-hour delay. Acceleration of advances and delays were also achieved with 5 hours of dim light per night, but effects depended on whether dim light was present in the first half, second half, or first and last quarters of the night. Both during phase shifting and steady-state entrainment, partially lit nights also produced strong positive and negative masking effects, as well as entrainment aftereffects in constant darkness. Thus, even in the presence of a strong zeitgeber, light that might be encountered under a natural nighttime sky potently modulates the circadian timing system of hamsters.

Key words circadian, entrainment, *Phodopus sungorus*, moonlight, masking, SCN

In mammals, light is the most important environmental cue that can reset the phase of the circadian pacemaker, thereby allowing entrainment to the environment both under natural conditions and following perturbations caused by abrupt time zone travel. Mediating this entrainment in mammals, at least in part, are melanopsin-expressing retinal ganglion cells that encode ambient lighting intensity and project directly to the central circadian pacemaker embodied in the suprachiasmatic nuclei (SCN) (Berson et al., 2002). Besides being anatomically distinct, this specialized circadian photoreceptive system is characterized by higher thresholds and longer integration times compared to the classic, image-forming visual system (Do et al., 2009). The response properties of the melanopsin-containing retinal

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ganglion cells are consistent with the idea that a primary function of the circadian entrainment mechanism is to distinguish day from night to assure proper phasing of the circadian timing system with respect to the solar day.

Laboratory studies of circadian rhythms commonly replace the natural pattern of illumination with alternating blocks of white light (e.g., 10-1000 lux) and complete darkness or dim red light putatively undetectable by the rodent retina. This convention, adopted for its simplicity, eliminates the gradual light intensity changes of twilight, demonstrated to be a potent modulator of the circadian timing system (Boulos et al., 2002; Danilenko et al., 2000; Kavanau, 1962; Laakso et al., 1988; Tang et al., 1999). Also omitted are the tonic and periodic illumination from the stars and moon. On a clear night, starlight can exceed 10^{-4} lux, and the full moon may generate approximately 10^{-1} to 10^{0} lux (Thorington, 1985) depending on geophysical factors (c.f. Erkert [1974], wherein a maximum of 0.3 lux was measured). Whereas the full moon illuminates the entire night, the first and last lunar quarters shine less intensely and for only half the night, setting and rising at approximately midnight, respectively.

In various laboratory settings, illumination of an intensity routinely encountered by a nocturnal mammal under a natural nighttime sky (~10^{-4}-10^{0} lux) can significantly alter the entrainment of circadian activity rhythms (Erkert, 2004; Erkert et al., 1976; Gorman et al., 2006). In Siberian (*Phodopus sungorus*) and Syrian (*Mesocricetus auratus*) hamsters, for example, the addition of dim nighttime illumination throughout the night (0.004-0.2 lux), compared to complete darkness, can facilitate adaptation to various exotic and natural light cycles and extend the range of entrainment (Evans et al., 2005; Evans et al., 2007; Gorman and Elliott, 2004; Gorman et al., 2003). Recently, we demonstrated that the addition of dim light at night hastened the re-entrainment time to a 4-hour phase advance or delay of the LD cycle by at least 38% in both hamster species (Evans et al., 2009). Because prior studies have provided dim illumination uniformly throughout the night, it is unclear whether there is a critical phase of sensitivity and, if so, when that occurs. Because dim light affects hamster rhythms in very diverse re-entrainment paradigms, we hypothesized that it would exert a permissive action on entrainment irrespective of circadian phase. On the other hand, the best documented circadian effects of brighter light, phase resetting, are strongly dependent on phase.

In addition to entraining the circadian pacemaker, light can exert acute effects on rhythmic outputs including suppression or stimulation of locomotor activity, termed negative and positive masking, respectively (Mrosovsky, 1999). In nature, the lunar cycle modulates expression of a number of behaviors of diverse species (Clarke, 1983; Daly et al., 1992; Kolb, 1992). For example, locomotor activity of owl monkeys (genus *Aotus*) is highest in full moonlight, producing a 24.8-hour rhythmicity under natural conditions (Erkert and Grober, 1986; Fernandez-Duque and Erkert, 2006). Analogously, movements of a prey species like the bushy-tailed wood rat, *Neotoma cinerea*, which is active on overexposed rocky outcrops, are suppressed by natural moonlight (Topping et al., 1999). In the laboratory as well, studies of masking in mice and Syrian hamsters have characterized the effects on wheel-running of discrete light pulses of varying irradiance (Mrosovsky, 1999; Mrosovsky et al., 1999; Redlin and Mrosovsky, 1999). Less understood is how nightly exposures to dim light modify ongoing activity rhythms in a laboratory rodent.

The present study was designed to test for the presence of a sensitive phase during which dim nighttime lighting (~0.1 lux) affects time zone resynchronization of Siberian hamsters. Hamsters under LD 14:10 received varying light exposures during the dark phase, including complete darkness for the entire night, dim light for the entire night, and dim light for only the first and last quarters, or the first or second half of the night. This study also evaluates whether illumination levels present under the night sky would produce masking and entrainment effects in hamster activity rhythms under a stable LD 14:10 cycle.

MATERIALS AND METHODS

Animals

A total of 54 young male Siberian hamsters (*Phodopus sungorus*) were born into an outbred colony maintained at UCSD since 1994 under a 14:10 LD schedule with approximately 100 lux during the photophase generated by 15-W fluorescent bulbs illuminating 3 cages each and with no dim illumination during the scotophase. Animals were provided with food (mouse chow 5015; Purina Mills, Gray Summit, MO) and water ad libitum. All procedures were approved by the UCSD Institutional Animal Care and Use Committee.

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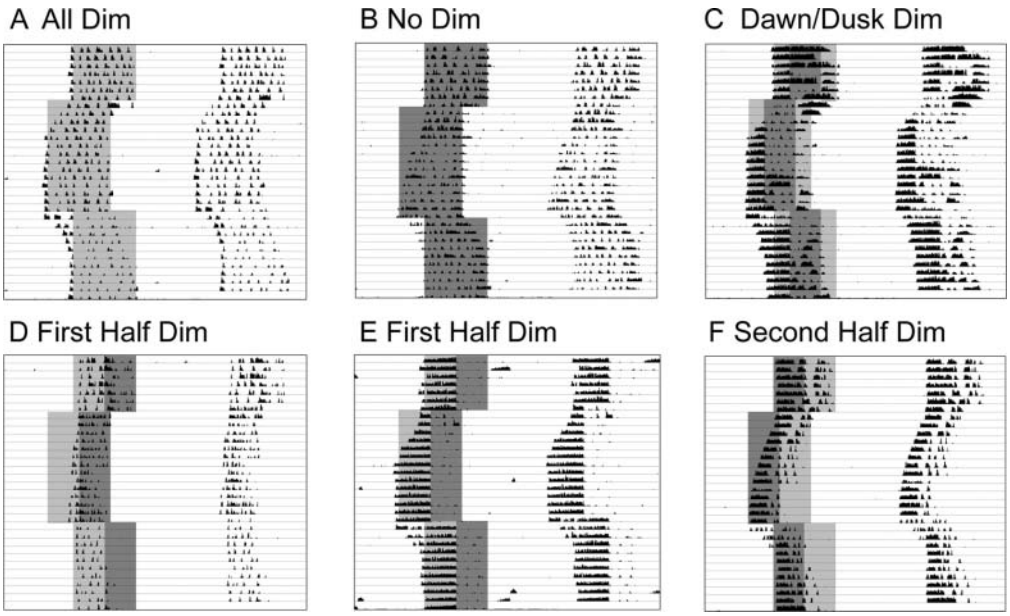

Figure 1. Double-plotted wheel-running activity records of male Siberian hamsters exposed first to a 4-hour phase advance and then a 4-hour delay under each of 5 conditions with 14 hours of daily light and 10 hours of dark or dim as follows: all dim (A), no dim (B), dawn/dusk (C), first half (D, E), and second half (F). Dim and dark scotophases are indicated with light and dark shading, respectively, on the left side only of the double plot. Actograms plot unmanipulated data scaled from 0 to the maximum value in 10 equal steps.

simulate a 4-hour eastward trip initiated at the light:dark (or light:dim) transition. Two weeks later, the light schedule was delayed by 4 hours, simulating a 4-hour trip west initiated at the light: dark transition (Fig. 1). A computer malfunction exposed animals to constant bright light for 2 days beginning 12 days after the phase delay. The experimental lighting conditions were restored for 35 days, after which all animals were transferred to constant darkness (no dim or bright light) for 16 days for assessment of the free-running wheel-running rhythm.

Procedure

At the start of the experiment, hamsters, 9 to 11 weeks of age, were singly housed in running-wheel cages (48 × 27 × 20 cm, 13-cm-diameter wheel) that were contained in ventilated, light-tight secondary enclosures each holding 3 cages. All hamsters remained on the LD 14:10 photoperiod with daytime illumination near 100 lux. Animals were randomly assigned to 1 of 5 groups that differed only in the nighttime light condition: no dim ($n = 12$) and all dim ($n = 12$) animals received no or constant nighttime illumination, respectively. Dawn/dusk animals ($n = 12$) received dim light for the first and last 2.5 hours of the night, whereas first half ($n = 9$) and second half ($n = 9$) hamsters received dim light for the first or last 5 hours of night, respectively. In the 4 groups receiving dim illumination, a single green LED (0.03 W; λ and half bandwidth equal to 560 and 23 nm, respectively) was mounted approximately 0.5 inches outside of each cage facing the running wheel, generating an average irradiance of 1.5×10^{-8} W/cm^2 measured from inside the running wheel by an IL1700 radiometer (International Light Inc., Newburyport, MA), equivalent to 0.1 lux.

After 26 days in LD 14:10, both daytime and nighttime lights were phase advanced by 4 hours to

Data Collection and Analysis

Wheel-running data were collected by VitalView data collection package (Mini Mitter; Respironics, Bend, OR), which compiled half-wheel revolutions in 6-minute bins. Actograms were prepared and analyzed with ClockLab software (Actimetrics, Willimette, IL).

Daily activity onsets and offsets were eye-fitted to actograms, and activity midpoint was derived as the average of these values. All values were converted to 3-day moving averages and expressed as the difference relative to each animal's baseline measures, defined as the 7-day average prior to the phase advance. A moving average was adopted to minimize the effect of individually determined activity offsets, which were quite variable, with the effect of rendering the re-entrainment data more monotonic. Re-entrainment time onsets and re-entrainment time midpoints were defined as the number of days for the corresponding 3-day moving average of each animal to shift 90% of the 4-hour light shift (e.g., 3.6 hours). Baselines were recalculated for the phase delay as the averages of activity onset and activity midpoint 3 days prior to the phase shift.

Masking of wheel-running by nighttime lighting conditions was evaluated by an observer blind to

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experimental condition as follows: actograms were printed in percentile and scaled format, regardless of condition, with 3 vertical lines that divided the 10-hour night into 4 equal 2.5-hour segments corresponding to the dim/dark transitions for the dim/dark and first half and second half groups. The rater assessed each actogram for abrupt activity intensity changes at the dividing lines following the first quarter night, half night, or three quarters night. Because activity levels change throughout the night and differed substantially between animals, a subjective method of rating masking was chosen over quantitative indices as these led to complicated and arbitrary parameterization that had not been independently validated. Masking was assessed during a span of 30 days that included baseline, phase advance, and phase delay portions of the experiment and separately over 30 days when conditions were unchanging prior to transfer to DD. Increases and decreases at those transitions were coded separately so as to permit determination of positive versus negative masking once the lighting conditions were revealed.

Two complementary analyses of re-entrainment were conducted (Evans et al., 2009). First, re-entrainment times were analyzed using a 1-way ANOVA with group as a between-subjects factor and followed by planned comparisons with the Student *t*-test. Second, daily activity onsets and activity midpoints were evaluated with repeated measures ANOVAs. As would be expected in a re-entrainment paradigm, both activity onset and activity midpoints changed over time with the phase shift producing highly significant main effects of time that are not reported here. Instead, main effects of group and time × group interactions, reflective of different re-entrainment patterns between groups, are reported. When these analyses revealed statistically significant effects, they were followed by repeated measures ANOVA to test for differences between all dim and no dim and among the 3 groups receiving 5 hours of nightly dim illumination. χ^2 analyses were used to evaluate the incidence of masking between groups.

One-way ANOVAs were also used to evaluate effects of dim nocturnal illumination on steady-state entrainment to the 14:10 LD cycle after phase advances and delays were complete. Using data from the last 10 days in LD 14:10, average values were calculated for activity counts (wheel rotations per minute), phase angle of entrainment (light offset minus activity onset), and activity duration (activity offset minus onset). In DD, activity counts, activity duration, and period, defined as least squares regression line through activity onsets on days 2 to 11, were assessed with 1-way ANOVAs.

Activity onsets of 1 hamster from first half and 2 from second half failed to advance by more than 2 hours in the first part of the experiment. Although more complete re-entrainment of these individuals might have occurred with a longer interval in unchanging LD 14:10, instead, the subsequent phase-delay data from these 3 animals with atypical baselines were excluded. Activity onset and midpoint baselines of the remaining subjects did not differ between the advance and delay portions of the experiment. Upon exposure to the phase delay, 2 additional animals from second half abruptly stopped running in their wheels, requiring that these individuals also be removed from that analysis.

RESULTS

Phase Advances and Delays

Representative actograms of individual animals in each experimental group during phase advances and delays are illustrated in Figure 1. Following a phase advance, both re-entrainment time midpoints and re-entrainment time onsets differed significantly between groups ($F_{4,49} = 5.35$, $p < 0.01$; $F_{4,49} = 2.71$, $p < 0.05$) (Fig. 2A, C). All dim and dawn/dusk groups re-entrained activity midpoints significantly faster than all other groups, but these 2 groups did not differ from one another. All dim and dawn/dusk groups did not differ from one another in re-entrainment time onsets, but both were significantly faster than second half animals.

Daily activity midpoints and daily activity onsets each occurred progressively earlier as expected following a phase advance (Figs. 1 and 3). Additionally, repeated measures ANOVA demonstrated a significant main effect of group for both measures ($F_{4,49} = 3.93$, $p < 0.01$; $F_{4,49} = 3.91$, $p < 0.01$, respectively) and showed a time × group interaction for activity midpoints ($F_{12,41} = 4.2$, $p < 0.001$). A similar interactive trend for activity onsets narrowly failed to reach statistical significance ($F_{12,41} = 1.8$, $p < 0.08$). For both measures, all dim and dawn/dusk groups advanced more or advanced earlier than other groups, whereas second half animals lagged behind. Post hoc repeated measures ANOVA confirmed the accelerated re-entrainment of all dim versus no dim hamsters for activity midpoints (group × time $F_{12,11} = 3.05$, $p < 0.05$) and a nonsignificant trend for activity onsets (group $F_{1,22} = 3.95$, $p < 0.06$). Likewise, the 3 groups receiving different patterns of 5 hours of nocturnal illumination differed significantly in activity midpoints (group

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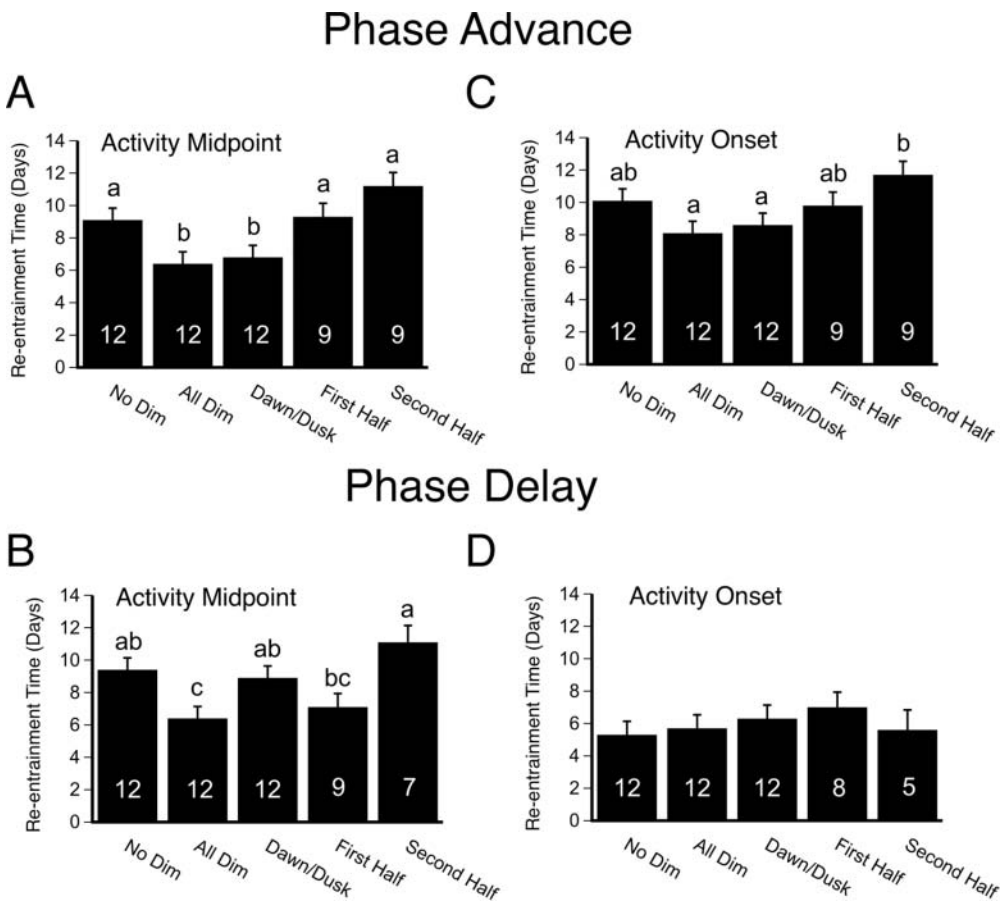

Figure 2. Mean (±SEM) re-entrainment time of activity midpoints (A, B) and activity onsets (C, D) following a 4-hour phase advance (A, C) and phase delay (B, D). Different letters indicate statistically significant differences between groups. Sample size is displayed in each data bar.

$F_{2,27} = 5.15$, $p < 0.05$; group × time $F_{12,17} = 2.98$, $p < 0.05$) and activity onsets (group $F_{2,27} = 4.28$, $p < 0.05$).

Following a phase delay, re-entrainment time midpoints differed significantly between groups ($F_{4,47} = 4.04$, $p < 0.01$) (Fig. 2B), but re-entrainment time onsets did not (Fig. 2D). For the former measure, all dim hamsters re-entrained significantly faster than the other groups except first half, which re-entrained faster than second half animals.

Daily activity midpoints and activity onsets occurred progressively later following a phase-delay shift. Activity onsets were unaffected by condition (Fig. 3D), but there was a main effect of group ($F_{4,47} = 3.24$, $p < 0.05$) and a time × group interaction for activity midpoints ($F_{11,40} = 4.2$, $p < 0.01$). A pairwise repeated measures ANOVA yielded a nonsignificant trend towards a difference between all dim and all dark midpoints ($F_{1,22} = 3.05$, $p < 0.10$). Differences between the 3 groups with 5 hours of dim illumination were reflected in a significant group × time interaction ($F_{11,16} = 3.1$, $p < 0.05$) and a nonsignificant trend towards a group effect ($F_{3,25} = 3.4$, $p < 0.10$).

Masking by Dim Light

Diverse patterns of apparent masking of wheel-running activity by dim nocturnal illumination are illustrated in selected actograms (Figs. 1 and 4). The determinations of masking corresponded precisely to actual dim:dark transitions (Fig. 5). That is, false-positive attributions of a masking effect were never recorded when there was no actual change in scotophase illumination. Both during phase shifting (Fig. 5A) and during steady-state entrainment to LD 14:10 (Fig. 5B), significant group differences in masking incidence were observed at each quarter of the night.

Positive masking (i.e., higher levels of activity in dim than in dark) occurred in approximately half of dawn/dusk animals in the first quarter of night (Figs. 1C, 4A, 5), whereas negative masking predominated at the end of night (Figs. 4F and 5). Animals exposed to dim during the first half of night could show either positive (Figs. 1E, 4C, 5) or negative masking (Figs. 4E and 5B). Conversely, negative masking was the more common response to dim illumination in the second half of night (50% animals) (Figs. 1F, 4B, 4D, 5), thereby generating largely similar activity patterns in the 2 groups illuminated for half of the night (c.f. Figs. 1E, 1F, 4B, 4C). In addition to individual differences between animals, there was considerable variability in masking responses in terms of day-to-day consistency (e.g., Fig. 1C demonstrates that on individual days, this hamster was not always more active in dim than in dark) and over the weeks of the experiment (e.g., Fig. 5).

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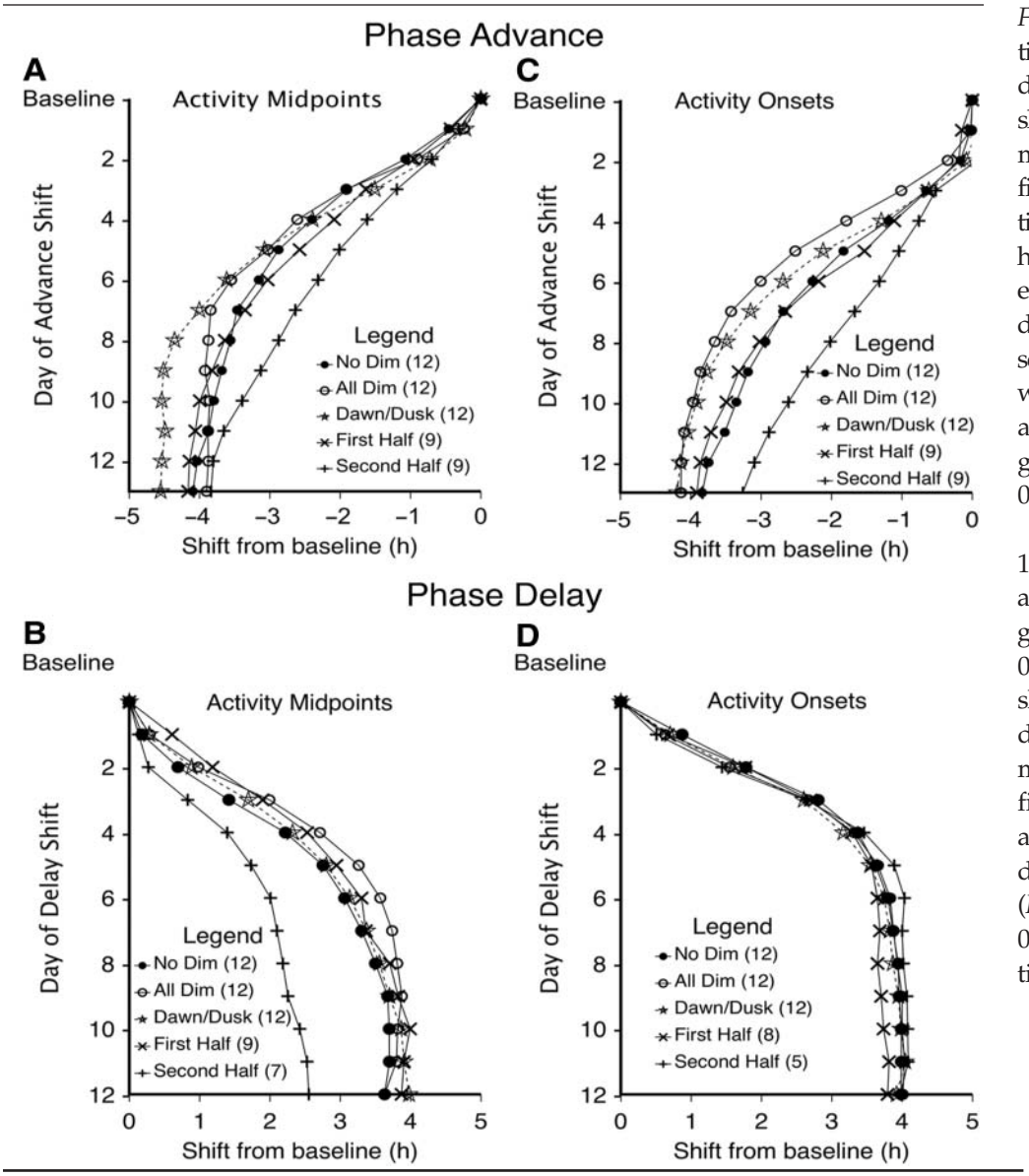

Figure 3. Mean activity midpoints (A, B) and activity onsets (C, D) following a 4-hour phase advance (A, C) and phase delay (B, D) expressed relative to each animal's preshift baseline.

In several cases (Fig. 4A, E, F), a sudden change in patterning of wheel-running behavior following transfer from LD 14:10 with dim light to DD further supports the interpretation of masking effects of dim light. In other cases, prior pacemaker effects of dim light are evidenced by the expression of a very short activity phase that expands gradually in DD (Fig. 4B, C).

Entrainment to LD 14:10 with Dim Nocturnal Illumination

Steady-state entrainment to LD 14: 10 after the phase delay differed by group as reflected by both activity duration and phase angle of entrainment ($F_{4,48} = 5.23$, $p < 0.01$; $F_{4,48} = 3.02$, $p < 0.05$, respectively) (Fig. 6B, C). Activity duration was significantly shorter in dawn/dusk animals than in all dim and first half groups. Additionally, the all dim group had earlier phase angles of entrainment than the dawn/dusk, no dim, and second half groups. There were no differences in activity counts between groups ($F_{4,49} = 0.96$, $p > 0.05$) (Fig. 6A).

In DD following LD 14:10, activity duration also differed between groups ($F_{4,46} = 6.65$, $p < 0.001$) (Fig. 6E), with shorter values in dawn/dusk and second half animals than in all dim and first half. Period and activity counts did not differ between groups ($F_{4,46} = 2.23$, $p > 0.05$; $F_{4,49} = 0.15$, $p > 0.05$, respectively).

DISCUSSION

The present study replicates and extends prior work demonstrating the facilitation of advancing and delaying phase shifts by dim nocturnal illumination (Evans et al., 2009). When presented throughout the night, dim illumination decreased the time to re-entrainment of the overt locomotor rhythm by 29% for advances and by 26% for delays of activity midpoints. Acceleration of advances and delays could be achieved also with shorter nightly exposures, but this dim-light enhancement of phase resetting depended on when in the night the dim light fell and the direction of the shift. Dim nighttime illumination also markedly influenced the nightly pattern of activity in LD 14:10 via both masking and entrainment effects. The results demonstrate potent effects of illumination comparable in intensity to that of moonlight on the functional organization of the

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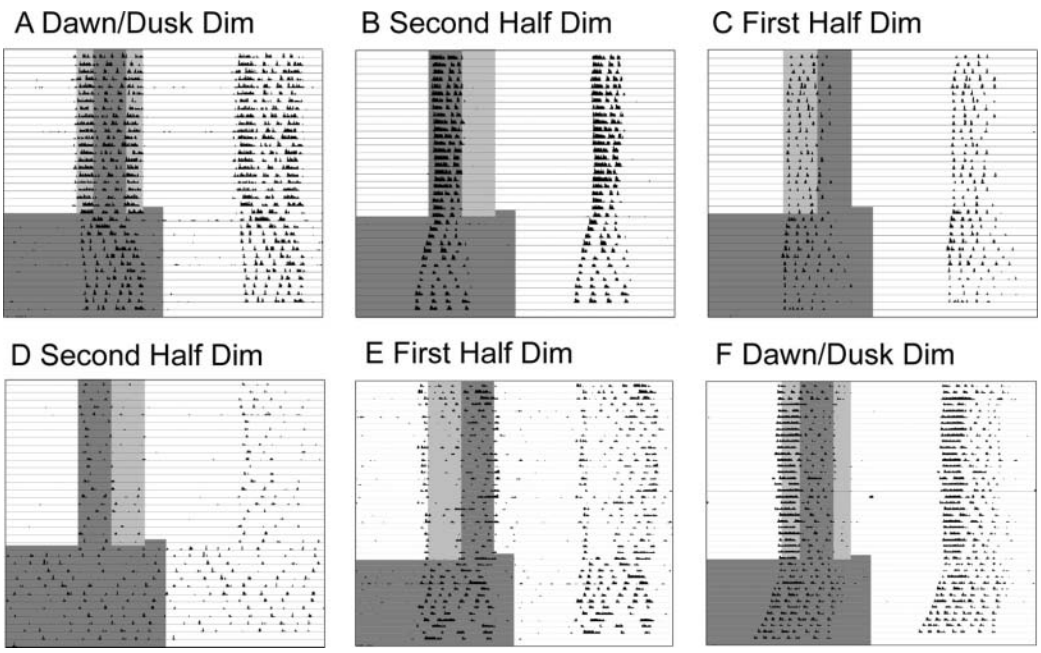

Figure 4. Double-plotted wheel-running activity records of hamsters under LD 14:10 with different nighttime conditions and in constant darkness. Conventions as in Figure 1.

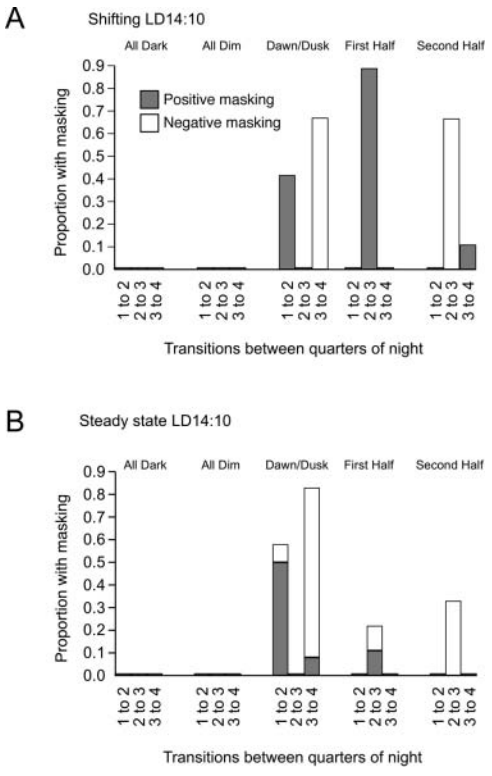

Figure 5. Assessed during 30 days of phase shifting (A) or steady-state entrainment to LD 14:10 (B), the proportion of animals in each experimental group showing positive (shaded bars) or negative masking (open bars) between first and second ($\chi^2_{4,54} = 19.3$, $p < 0.001$; $\chi^2_{8,54} = 28.2$, $p < 0.001$), second and third ($\chi^2_{8,54} = 80.1$, $p < 0.001$; $\chi^2_{8,54} = 17.1$, $p < 0.05$), and third and fourth quarter ($\chi^2_{8,54} = 32.5$, $p < 0.001$; $\chi^2_{8,54} = 43.0$, $p < 0.001$), respectively.

circadian timing system of hamsters. Comparable light intensities have demonstrated relevance to circadian timing in fruit flies, suggesting perhaps a broad taxonomic significance of natural nighttime illumination (Bachleitner et al., 2007; Rieger et al., 2009).

Because dim light of the sort used here was previously shown to markedly alter entrainment in a great variety of conventional and idiosyncratic lighting conditions (Evans et al., 2005; Evans et al., 2007; Gorman and Elliott, 2004; Gorman et al., 2003), we hypothesized that it might be having a nonspecific action on the pacemaker independent of its circadian phase of application. This hypothesis must be clearly rejected as 3 of the 4 phase-shifting measures reveal differences between the 3 groups receiving 5 hours of dim illumination scheduled at different phases. Moreover, the one measure not showing any such effect, activity onsets following phase delays, also did not differ between no dim and all dim groups. Thus, by whatever means that dim light at night facilitates re-entrainment to phase-shifted light cycles, it does so differentially across the night.

Beyond this conclusion, a general principle that explains the specific patterns of re-entrainment under the three 5-hour conditions does not readily emerge (e.g., that dim light at different times of night selectively facilitates advances versus delays or that shifting into dim portions of the night is faster than shifting into dark portions). Whereas dim light at the beginning and end of night (i.e., dawn/dusk) accelerated phase advances, it did not accelerate delays; the opposite was true for light in the first half of night. This lack of transparency may reflect the fact that in the present experimental design, dim light necessarily falls at very different circadian phases before and immediately after a phase shift and continues to fall at different phases as the shift is realized.

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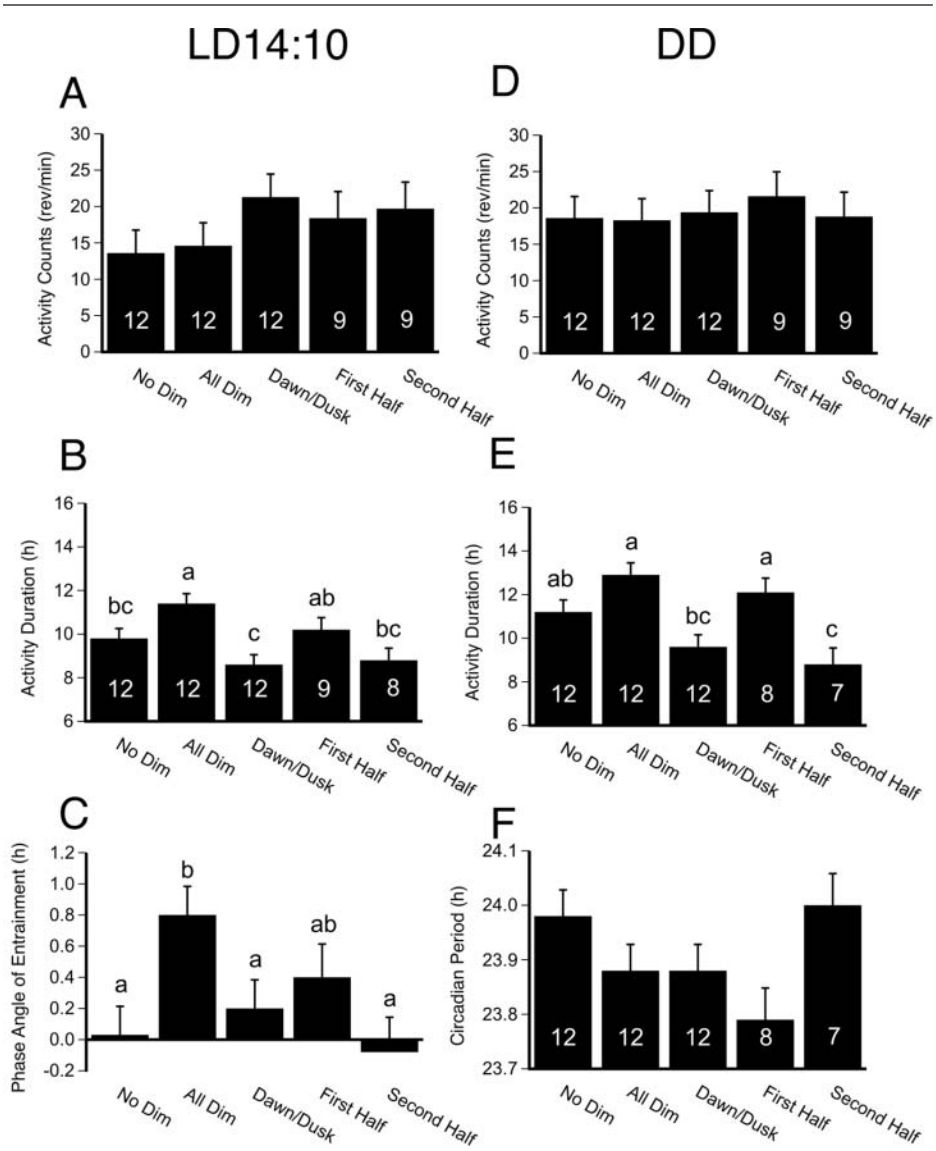

Figure 6. Mean (±SEM) activity counts (A, D), activity duration (B, E), phase angle of entrainment (C), and circadian period (F) of hamsters in LD 14:10 (A-C) or DD (D-F). Sample size in C is same as in B. Conventions as in Figure 2.

For example, when animals in the first half group are exposed to an abrupt 4-hour phase advance, the dim light may initially fall nearly or entirely prior to the subjective night (Fig. 1D). Only as the phase shift is completed would it coincide again with the first half of subjective night. This interpretation is supported by the finding of a prior study that there is a critical role of dim light on cycles following the zeitgeber shift (Evans et al., 2009).

Unexpected in the present study was the finding of both negative and positive masking to the same dim light stimulus in different individuals (Fig. 4A, F). In mice, a switch from positive to negative masking occurs with increasing light intensity, likely mediated by distinct photoreceptor mechanisms (Mrosovsky and Thompson, 2008). In the present experiment, the standardized placement of the dim light source relative to the cage would have produced uniform intensities between cages, but behavioral or physiological factors could have modulated the effective illumination received by individual animals. Because the switch from positive to negative masking in mice occurs gradually over several orders of magnitude of irradiance, we consider it more likely that individual differences in masking behavior arise postretinally. Compared to laboratory rodents, in which masking has been most extensively studied, the outbred Siberian hamster has considerably more genetic variability to underlie individual variation in such responses. White-footed mice as well exhibit pronounced individual differences in their directional orientation of wheel-running behavior to a light source simulating the moon (Kavanau, 1967).

Besides individual differences in response to identical stimuli, the same dim illumination clearly produced different effects on the locomotor activity rhythm at different phases of the night, just as it did on rate of re-entrainment. Indeed, in Figure 4F, the same animal appears to show positive masking at the beginning of the night and negative masking at the end. The same is true for many other animals in the dawn/dusk group, although an entrainment effect of late night dim illumination cannot be ruled out (see below). Clearer is that positive masking predominates when dim is present in the first half of the night, whereas negative masking is the modal response to dim light in the latter half. Daily rhythms in mask-

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ing responses to dark and bright light have been previously reported in Syrian hamsters (Aschoff and von Goetz, 1988; Redlin and Mrosovsky, 1999) and other species (Aschoff and von Goetz, 1989), but to our knowledge, no study has yet documented a change in masking direction as a function of time.

A curious finding was that compared to all dim, dawn/dusk groups produced a 0.6-hour phase lag in activity onset and a nearly 3-hour shortening of activity duration both in LD 14:10 and in DD, the latter aftereffects obviously not due to masking. Post hoc, some of the instances coded as negative masking by light in the last quarter of night of the dawn/dusk group (Fig. 5A, B) instead appear to represent an advanced phase of entrainment of activity offset, although not in all cases (Fig. 4F). The distinction between the all dim and dawn/dusk groups is the presence or absence of complete darkness in the middle of the night. Thus, 5 hours of total dark appears to render some animals sufficiently sensitive to dim light that they may be entrained by its onset near the end of night. A comparable entrainment action was seen in some second half animals (Fig. 4B) but was too rare to substantially affect the mean values of activity onset and activity duration. Entrainment effects of light near this intensity have been reported in other rodents (reviewed in Erkert [2004]), and much dimmer light can entrain rhythms of bats (Erkert, 2004), but these effects were observed against a background of complete darkness. That they occur also in the presence of a bright photophase establishes that variations in illumination throughout the night could have functional importance for circadian entrainment under natural conditions that include a wide range of light intensities. In a review of masking and parametric actions of light, Aschoff (1999) warned not to "overemphasize the role played by non-parametric entrainment," referring to the idea that only discrete phase-shifting actions of bright light at dawn and dusk dictate parameters of circadian entrainment. The present results validate this concern, as do recent studies of *Drosophila* (Bachleitner et al., 2007; Rieger et al., 2009).

Under natural conditions, light intensity changes gradually through dawn and dusk, although light sampling behavior of animals with recourse to burrows implies that effective illumination levels may change rapidly. According to field researchers, Siberian hamsters can be tracked visually in the grass of the Siberian steppe (Wynne-Edwards et al., 1992) and thus might be expected to experience nighttime light at least as bright as provided in this study. Although we did not simulate the spectral or the gradually moving pattern of moonlight with respect to the solar day, the first half and second half groups share features with the first and last quarter moons, setting and rising at midnight. Whether entrainment and masking effects would persist with incorporation of twilights, self-selected light sampling, and complete simulation of moonlight remains to be determined.

By what mechanism does dim light alter entrainment and masking? At the photoreceptor level, the highly sensitive rods are a likely candidate given their established relationship to positive masking in mice (Mrosovsky and Thompson, 2008), although an action spectrum study in progress will test this hypothesis more rigorously. Regardless, dim light could affect the pacemaker directly via the retinohypothalamic tract (RHT), via polysynaptic photic pathways from geniculate or midbrain structures (Morin et al., 2003), or more indirectly still through feedback effects of locomotor activity (Bobrzynska and Mrosovsky, 1998). Arguing against the latter possibility, both current and prior studies establish dim facilitation of re-entrainment in the absence of any effect on wheel-running intensity (Evans et al., 2009). Further, in other contexts in which dim light did increase wheel-running, experimental manipulations determined that the wheel-running was not a causal factor (Evans et al., 2005). Additional insight into mechanism might be gained by considering the convergent effects of dim illumination (Evans et al., 2005; Evans et al., 2007; Evans et al., 2009; Gorman et al., 2003; Gorman et al., 2005). A working hypothesis to be further tested is that dim light modulates entrainment by affecting coupling within the central pacemaker (Gorman and Elliott, 2004; Gorman et al., 2006; Meijer et al., 1990).

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REFERENCES

Aschoff J (1999) Masking and parametric effects of high-frequency light-dark cycles. Jap J Physiol 49:11-18.

Aschoff J and von Goetz C (1988) Masking of circadian activity rhythms in hamsters by darkness. J Comp Physiol [A]-Sens Neural Behav Physiol 162:559-562.

Aschoff J and von Goetz C (1989) Masking of circadian activity rhythms in canaries by light and dark. J Biol Rhythms 4:29-38.

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Bachleitner W, Kempinger L, Wulbeck C, Rieger D, and Helfrich-Forster C (2007) Moonlight shifts the endogenous clock of *Drosophila melanogaster*. Proc Natl Acad Sci U S A 104:3538-3543.

Berson DM, Dunn FA, and Takao M (2002) Phototransduction by retinal ganglion cells that set the circadian clock. Science 295:1070-1073.

Bobrzynska KJ and Mrosovsky N (1998) Phase shifting by novelty-induced running: activity dose-response curves at different circadian times. J Comp Physiol [A] 182:251-258.

Boulos Z, Macchi MM, and Terman M (2002) Twilights widen the range of photic entrainment in hamsters. J Biol Rhythms 17:353-363.

Clarke JA (1983) Moonlights influence on predator prey interactions between short-eared owls (*Asio flammeus*) and deermice (*Peromyscus maniculatus*). Behav Ecol Sociobiol 13:205-209.

Daly M, Behrends PR, Wilson MI, and Jacobs LF (1992) Behavioural modulation of predation risk moonlight avoidance and crepuscular compensation in a nocturnal desert rodent *Dipodomys merriami*. Anim Behav 44:1-9.

Danilenko KV, Wirz-Justice A, Krauchi K, Weber JM, and Terman M (2000) The human circadian pacemaker can see by the dawn's early light. J Biol Rhythms 15:437-446.

Do MT, Kang SH, Xue T, Zhong H, Liao HW, Bergles DE, and Yau KW (2009) Photon capture and signalling by melanopsin retinal ganglion cells. Nature 457:281-287.

Erkert HG (1974) Der einfluß des mondlichtes auf die Aktivitätsperiodik nachtaktiver Säugetiere. Oecologia 14:269-287.

Erkert HG (2004) Extremely low threshold for photic entrainment of circadian activity rhythms in molossid bats (*Molossus molossus*; Chiropter – Molossidae). Mammal Biol 69:361-374.

Erkert HG, Bay FA, and Kracht S (1976) Zeitgeber induced modulation of activity patterns in nocturnal mammals (*Chiroptera*). Experientia 32:560-562.

Erkert HG and Grober J (1986) Direct modulation of activity and body temperature of owl monkeys (*Aotus lemurinus griseimembra*) by low light intensities. Folia Primatol 47:171-188.

Evans JA, Elliott JA, and Gorman MR (2005) Circadian entrainment and phase resetting differ markedly under dimly illuminated versus completely dark nights. Behav Brain Res 162:116-126.

Evans JA, Elliott JA, and Gorman MR (2007) Circadian effects of light no brighter than moonlight. J Biol Rhythms 22:356-367.

Evans JA, Elliott JA, and Gorman MR (2009) Dim nighttime illumination accelerates adjustment to timezone travel in an animal model. Curr Biol 19:R156-157.

Fernandez-Duque E and Erkert HG (2006) Cathemerality and lunar periodicity of activity rhythms in owl monkeys of the Argentinian Chaco. Folia Primatologica 77:123-138.

Gorman MR and Elliott JA (2004) Dim nocturnal illumination alters coupling of circadian pacemakers in Siberian hamsters, Phodopus sungorus. J Comp Physiol A 180: 631-639.

Gorman MR, Elliott JA, and Evans JA (2003) Plasticity of hamster circadian entrainment patterns depends on light intensity. Chronobiol Int 20:233-248.

Gorman MR, Evans JA, and Elliott JA (2006) Potent circadian effects of dim illumination at night in hamsters. Chronobiol Int 23:245-250.

Gorman MR, Kendall M, and Elliott JA (2005) Scotopic illumination enhances entrainment of circadian rhythms to lengthening light:dark cycles. J Biol Rhythms 20:38-48.

Kavanau JL (1962) Twilight transitions and biological rhythmicity. Nature 194:1293-1295.

Kavanau JL (1967) Behaviour of captive white-footed mice. Science 155:1623-1639.

Kolb HH (1992) The effect of moonlight on activity in the wild rabbit *Oryctolagus cuniculus*. J Zool 228:661-665.

Laakso ML, Porkka-Heiskanen T, Alila A, Peder M, and Johansson G (1988) Twenty-four-hour patterns of pineal melatonin and pituitary and plasma prolactin in male rats under 'natural' and artificial lighting conditions. Neuroendocrinology 48:308-313.

Meijer JH, Daan S, Overkamp GJF, and Hermann PM (1990) The 2-oscillator circadian system of tree shrews (*Tupaia belangeri*) and its response to light and dark pulses. J Biol Rhythms 5:1-16.

Morin LP, Blanchard JH, and Provencio I (2003) Retinal ganglion cell projections to the hamster suprachiasmatic nucleus, intergeniculate leaflet, and visual midbrain: bifurcation and melanopsin immunoreactivity. J Comp Neurol 465:401-416.

Mrosovsky N (1999) Masking: history, definitions, and measurement. Chronobiol Int 16:415-429.

Mrosovsky N, Foster RG, and Salmon PA (1999) Thresholds for masking responses to light in three strains of retinally degenerate mice. J Comp Physiol [A] 184:423-428.

Mrosovsky N and Thompson S (2008) Negative and positive masking responses to light in retinal degenerate slow (rds/rds) mice during aging. Vis Res 48: 1270-1273.

Redlin U and Mrosovsky N (1999) Masking of locomotor activity in hamsters. J Comp Physiol [A] 184:429-437.

Rieger D, Wulbeck C, Rouyer F, and Helfrich-Forster C (2009) Period gene expression in four neurons is sufficient for rhythmic activity of *Drosophila melanogaster* under dim light conditions. J Biol Rhythms 24:271-282.

Tang IH, Murakami DM, and Fuller CA (1999) Effects of square-wave and simulated natural light-dark cycles on hamster circadian rhythms. Am J Physiol 276:R1195-1202.

Thorington L (1985) Spectral, irradiance, and temporal aspects of natural and artificial light. Ann N Y Acad Sci 453:28-54.

Topping MG, Millar JS, and Goddard JA (1999) The effects of moonlight on nocturnal activity in bushy-tailed wood rats (*Neotoma cinerea*). Can J Zool 77:480-485.

Wynne-Edwards KE, Surov AV, and Telitzina AY (1992) Field studies of chemical signalling: direct observations of dwarf hamsters (*Phodopus*) in Soviet Asia. In *Chemical Signals in Vertebrates VI*, Doty RL and Müller-Schwarze D, eds, pp 485-491. New York: Plenum.

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