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ORIGINAL PAPER

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Dim nocturnal illumination alters coupling of circadian pacemakers in Siberian hamsters, *Phodopus sungorus*

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Abstract The circadian pacemaker of mammals comprises multiple oscillators that may adopt different phase relationships to determine properties of the coupled system. The effect of nocturnal illumination comparable to dim moonlight was assessed in male Siberian hamsters exposed to two re-entrainment paradigms believed to require changes in the phase relationship of underlying component oscillators. In experiment 1, hamsters were exposed to a 24-h light-dark-light-dark cycle previously shown to split circadian rhythms into two components such that activity is divided between the two daily dark periods. Hamsters exposed to dim illumination (<0.020 lx) during each scotophase were more likely to exhibit split rhythms compared to hamsters exposed to completely dark scotophases. In experiment 2, hamsters were transferred to winter photoperiods (10 h light, 14 h dark) from two different longer daylengths (14 h or 18 h light daily) in the presence or absence of dim nighttime lighting. Dim nocturnal illumination markedly accelerated adoption of the winter phenotype as reflected in the expansion of activity duration, gonadal regression and weight loss. The two experiments demonstrate substantial efficacy of light intensities generally viewed as below the threshold of circadian systems. Light may act on oscillator coupling through rod-dependent mechanisms.

Keywords Oscillator photoperiod coupling · Splitting entrainment

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Abbreviations α: activity duration · DD: constant dark or dim · E: evening oscillator · ETV: estimated testis volume · LDLD: light-dark-light-dark cycle · LED: light emitting diode · M: morning oscillator · SCN: suprachiasmatic nuclei · τ: free-running period

Introduction

Circadian rhythms of many mammalian species exhibit seasonally changing patterns of entrainment (Daan and Aschoff 1975). In hamsters, for example, various circadian markers of "subjective night" (e.g., increased locomotor activity, elevated pineal melatonin secretion, light-induced phase-resetting) are programmed for a longer fraction of the daily cycle during entrainment to short winter daylengths than they are under long summer daylengths (Pittendrigh et al. 1984; Illnerova 1991; Elliott and Tamarkin 1994). When hamsters are transferred from short to long photoperiods, the resetting actions of the light falling during subjective night result in rapid entrainment into the summer-typical circadian waveform (Illnerova 1991; Sumova et al. 1995). Following transfer from long to short photoperiods, in contrast, the expansion of subjective night proceeds gradually, and at different rates depending on the phasing of the old and new photoperiods (Hoffmann et al. 1986; Illnerova et al. 1986; Gorman et al. 1997). As the circadian-driven rhythm of pineal melatonin secretion is the proximate stimulus inducing seasonal changes in reproduction, metabolism and thermoregulatory behaviors (Goldman 2001), changes in these latter traits are likewise manifest at different rates depending on the nature of the photoperiod transfer (Hoffmann et al. 1986; Illnerova et al. 1986; Gorman et al. 1997). In the highly photoperiodic Siberian hamster, moreover, a fraction of animals fails altogether to adopt the typical short-day entrainment pattern and thus never exhibits the winter-adapted phenotype (Puchalski and Lynch 1986; Prendergast and Freeman 1999).

The photoperiodic regulation of circadian rhythms has been fruitfully modeled as the consequence of two interacting circadian oscillators that are principally entrained by the resetting action of light at dawn and dusk, respectively (Pittendrigh and Daan 1976; Daan and Berde 1978; Elliott and Tamarkin 1994; Gorman et al. 1997). Termed evening (E) and morning (M) oscillators, these circadian components may adopt a range of phase angles to regulate the circadian waveform. Little is known about the interaction, or coupling, between these oscillators, despite the central role of this topic in circadian modeling (Daan and Berde 1978; Oda et al. 2000; Kunz and Achermann 2003). The variability in the rate of entrainment to short photoperiods and the failure of photo-nonresponsive hamsters to lengthen activity duration under winter photoperiods likely derive from properties of coupling between oscillators (Illnerova et al. 1986; Gorman et al. 1997).

Recently, novel temporal relationships between component circadian oscillators have been described in a phenomenon referred to as "behavioral decoupling" or "splitting." Following appropriately timed repeated exposure to novel running wheels (Mrosovsky and Janik 1993; Gorman and Lee 2001) or to 24 h light-dark-light-dark (LDLD) cycles (Gorman 2001; Evans and Gorman 2002; Gorman et al. 2003), hamsters may express markers of subjective night in two discrete intervals daily. This split pattern is readily entrained to LDLD cycles during which robust locomotor activity is expressed in each of the two daily scotophases. Studies with skeleton photoperiods suggest that the pattern reflects true entrainment of two separate circadian oscillators in a novel phase relationship (Gorman and Elliott 2003). Although these two components may be separately entrained by light for an indefinite period, when released into constant dark or dim (DD) the components re-join within several cycles under the influence of strong oscillator interactions (Mrosovsky and Janik 1993; Gorman 2001; Gorman and Elliott 2003).

In rodents, circadian phototransduction depends on the retina, but can occur in the absence of both rods and cones (Freedman et al. 1999). High threshold, intrinsically photosensitive retinal ganglion cells project directly to the dominant circadian pacemaker, the suprachiasmatic nuclei (SCN) (Hattar et al. 2002). As the photic threshold for inducing neuronal firing in these cells resembles that for circadian phase-shifting, these cells are likely candidates for primary sensory afferents to the circadian system (Berson 2003). Nevertheless, illumination intensity far below that detectable by these cells has been shown to alter circadian rhythms. In a recent study of Syrian hamsters, *Mesocricetus auratus*, light of lower intensity than full moonlight facilitated adoption of a split entrainment pattern to a LDLD cycle, suggesting that dim illumination may alter oscillator coupling (Gorman et al. 2003). The present study assesses whether this effect of dim nocturnal illumination is common to other species that exhibit rhythm splitting. More significantly, this study seeks convergent evidence for an influence of dim light on oscillator coupling by assessing circadian re-entrainment following transfer from long to short photoperiods. Two versions of this re-entrainment paradigm were selected on the basis of the sluggish photoperiodic response following transfer to short daylengths. First, transfer from long to short daylengths is significantly retarded if only the time of lights off is altered. Second, pre-treatment with very long daylengths (e.g., 18L) induces a substantial fraction of hamsters to maintain a long-day entrainment state despite prolonged exposure to short photoperiods. The results of these experiments establish a strong influence of dim scotophase illumination on circadian re-entrainment, with functional consequences for the photoperiodic regulation of reproduction and body weight.

Methods and materials

Male Siberian hamsters, *Phodopus sungorus*, were colony-bred from stock originally supplied by Bruce Goldman (University of Connecticut, Storrs, Conn., USA). Hamsters were born and group housed from weaning in 14 h light and 10 h dark daily (LD14:10; lights off 2000–0600 hours Pacific Standard Time, PST) with food (Purina Chow no. 5015) and water provided ad libitum. Except as noted below, hamsters were housed in clear polypropylene cages (27×16×13 cm, two to four hamsters per cage) on corncob bedding. Temperature was maintained at 22 ± 2°C. In the colony room, no illumination was provided during the scotophase, and light intensity was 50–100 lx during the photophase.

Experiment 1: does dim nocturnal illumination facilitate rhythm splitting?

Male hamsters, 5–8 weeks old, were transferred from group housing in LD14:10 to individual opaque polypropylene cages (27 cm×20 cm×15 cm) equipped with running wheels (17 cm diameter). The transfer occurred at 1000 hours PST, which began a 5-h scotophase of a new LDLD 7:5:7:5 light regimen. In the new cages, photophase illumination was produced by 4-W fluorescent lamps that generated an illuminance of 100–150 lx at the cage floor. For scotophase illumination, hamsters were randomly assigned to one of two conditions: during each scotophase, they received either complete darkness (dark group; $n = 10$) or dim illumination (dim group; $n = 10$). Dim illumination was provided by a single green light emitting diode (LED, peak wavelength of 560 nm) generating a cage floor illuminance < 0.02 lx at the brightest location in the cage (model 371 optical power meter; UDT Instruments, Baltimore, Md., USA). This corresponds to a maximum irradiance of 10^{10} photons cm^{-2} s^{-1}. The cage bedding was changed at 2-week intervals during the 1st hour of the daytime scotophase. This procedure involved brief exposure (1–2 min) to dim

red illumination (<1 lx, >600 nm). After 33 days in the LDLD cycle, the fluorescent lights were permanently extinguished, and free-running rhythms were monitored for an additional 2 weeks.

Analysis

Wheel running counts were compiled into 6-min bins by DataQuestIII software (Mini Mitter, Bend, Ore., USA) and actograms were prepared in ClockLab (Actimetrics, Evanston, Ill., USA). Following previous studies (Gorman and Elliott 2003), rhythms were considered "split" if threshold activity levels (5 counts min^{-1}) were exceeded in both of the twice daily scotophases for six successive bins on 7 days of any 14-day interval. In practice, there was no ambiguity about whether animals should be considered split or not. Chi-squared statistics were used to assess group differences in the proportion of animals with split rhythms.

Experiment 2: does dim nocturnal illumination facilitate re-entrainment?

Male hamsters, 12–16 weeks old, were transferred from LD 14:10 to LD 18:6 (lights off 2000 hours PST). For half of the animals (dim group; $n=17$), a green LED placed approximately 20 cm from each cage generated a "darkness" illuminance level <0.02 lx ($<10^{10}$ photons cm^{-2} s^{-1}) at the cage floor. The scotophase was completely dark for the remaining hamsters (dark group; $n=18$). For all hamsters, 15-W fluorescent lamps provided photophase light levels of 100–200 lx. After 23 days in LD 18:6, the photoperiod was shortened to LD 10:14 (lights off 1600 hours PST), but animals remained in their respective dark or dim conditions. At that time, 13 age-matched hamsters were transferred directly from the LD 14:10 colony room to individual cages and exposed to the same LD 10:14 conditions ($n=7$ in dim; $n=6$ in dark).

Body weights were measured at the beginning of exposure to LD 10:14 (week 0) and at 4-week intervals. At weeks 4 and 8, hamsters were lightly anesthetized with isoflurane vapors (Aerane, Fort Dodge, Iowa, USA), and the length and width of the left testis were measured externally with calipers. The product of testis length and the squared testis width yields a reliable index of testis size (estimated testis volume, ETV) (Gorman and Zucker 1997). At week 8, hamsters were transferred to larger clear cages (48×27×20 cm) equipped with running wheels (17 cm diameter). After five nights, the LEDs were extinguished for the dim groups, and all animals were monitored under identical conditions (LD 10:14, completely dark scotophases) for an additional 4 days.

Except in the final days of the study, locomotor activity rhythms were monitored with passive infrared motion detectors (Coral Plus; Visonic, Bloomfield, Conn., USA) mounted on filter tops. Movement under the recording area triggered the closure of a relay, and the number of such closures was recorded with the VitalView data collection package (Mini Mitter, Bend, Ore., USA) and compiled into 6-min bins.

Analysis

Actograms were prepared, and two independent observers blind to the experimental conditions drew lines through activity onsets and activity offsets over successive 7-day intervals beginning with the transfer to LD 10:14. The mean value of each line was taken as the activity onset and offset, respectively, for that week, and activity duration (α) was calculated as the difference between these two values. On a set of eight representative actograms, the correlation between the two raters exceeded 0.98 for each of these three variables. To evaluate apparent arrhythmicity in some animals, chi-squared periodograms were calculated from 20 to 28 h, $\alpha=0.001$ (ClockLab), using the final 10 days of motion-detector data.

For wheel-running data, activity onset and offset were defined as the first and last time points in the scotophase that levels exceeded 5 counts min^{-1} and were sustained for three consecutive 6-min bins. Following prior studies of photoresponsiveness with longer exposure to short photoperiods (Gorman and Zucker 1997; Prendergast and Freeman 1999), the circadian system was considered to be photononresponsive if $\alpha<8.0$ h as measured by motion detectors in week 8 of LD 10:14 or by running wheels in the following week.

Statistics

For the 8 weeks in LD 10:14, activity onsets, offsets and durations as determined from motion detector data were assessed by repeated measures analysis of variance (ANOVA; Statview 5.0, SAS Institute, Cary, N.C., USA). Main effects of "condition" reflect differences between dim- and dark-exposed hamsters averaged over the entire 8-week interval. Main effects of "time" reflect temporal changes without regard to lighting condition. The time×condition interaction indexes different patterns of change over time for the dim- and dark-exposed groups. Comparisons of dim- and dark-exposed hamsters at individual time points were conducted with Student's *t*-tests with a Bonferroni correction for multiple comparions (i.e., reported as $P<0.05$ if uncorrected $P<0.00625$). Group differences in the incidence of nonresponsiveness were assessed with chi-squared tests.

Results

Experiment 1: rhythm splitting

As in previous studies, Siberian hamsters entrained to LDLD cycles with one of two distinct patterns. With

completely dark scotophases, a majority of hamsters expressed robust locomotor activity in only one of the two daily scotophases. These hamsters typically exhibited some evidence of negative masking by light onset (Fig. 1A). Other hamsters split activity into two daily components, one associated with each of the two daily scotophases (Fig. 1B, C). In the first 2 weeks, significantly more hamsters exposed to dim scotophases entrained with this split activity pattern than did those given complete darkness (Fig. 2; $P < 0.05$). In the following 2 weeks, one additional hamster from each group adopted this entrainment pattern (e.g., Fig. 1B). Upon exposure to constant conditions, animals with split rhythms produced two distinct activity components for at least two cycles, but eventually the components joined to produce a single longer interval of activity. Hamsters entraining with unsplit rhythms exhibited a gradual lengthening of activity duration after transfer to DD (Fig. 1).

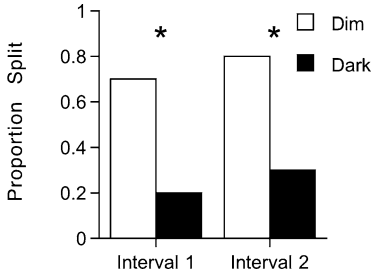


Fig. 2 Proportion of hamsters adopting the split entrainment pattern with completely dark or dimly illuminated scotophases of a light-dark-light-dark (LDLD) 7:5:7:5 photocycle. Data for the first and second 2-week intervals in LDLD are presented. *Asterisk* indicates that the proportion of split hamsters differs between groups (χ^2; $P < 0.05$). Sample size is 10 per group

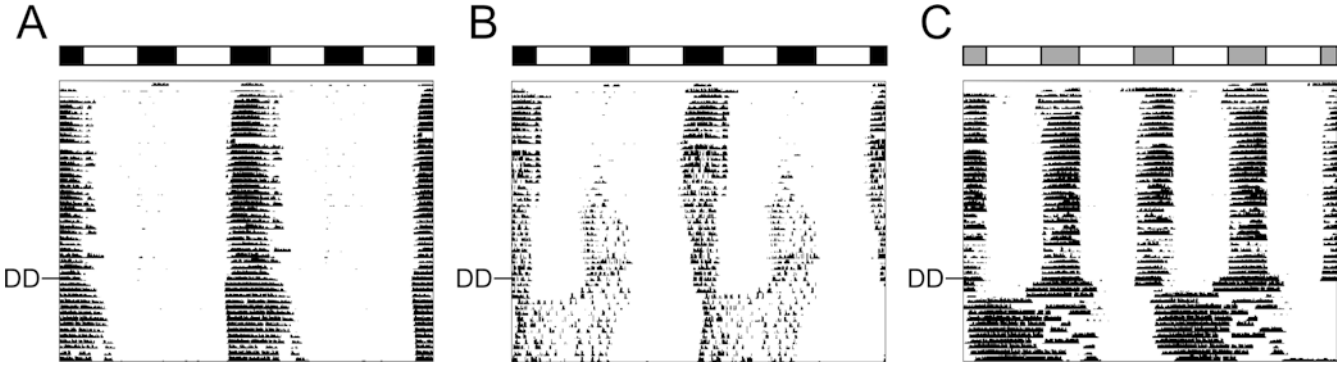
Experiment 2: winter re-entrainment

All but nine hamsters showed clear locomotor rhythms with readily discernible activity onsets and offsets (Fig. 3A–D). Following transfer to LD10:14, most hamsters exhibited gradual lengthening of activity duration accomplished via advances of activity onset, delays in activity offset, or both. Atypical activity rhythms were present in 8 hamsters previously exposed to LD18:6 and in one hamster from LD14:10. These nine hamsters are excluded from the primary analyses, but are considered separately below.

Following transfer from LD 18:6 to LD 10:14, mean activity onsets of dim-exposed hamsters progressively advanced whereas those of dark-exposed hamsters initially advanced but then delayed, generating a significant time×condition interaction ($P < 0.001$). Considered over the entire 8-week interval, activity onsets were significantly earlier for dim-exposed hamsters ($P < 0.05$). At individual time points, activity onsets of dim hamsters were significantly earlier at weeks 7 and 8 (Fig. 4; $P < 0.05$). Activity offsets occurred later as the study progressed ($P < 0.001$), but there was no significant effect of lighting condition and no significant time×condition interaction. Activity duration expanded over time ($P < 0.001$) with greater effects observed in dim-exposed hamsters ($P < 0.001$). Dim-exposed hamsters displayed longer activity durations at week 2 and from week 5 onwards (Fig. 4A).

Among hamsters transferred from LD 14:10 to LD 10:14, activity onsets progressively advanced (Fig. 4B; $P < 0.001$), significantly more in dim scotophases than in dark ($P < 0.05$). At no single time point, however, did activity onset differ significantly between groups. Likewise, activity offsets progressively delayed over the 8 weeks of the experiment ($P < 0.001$). Dim-exposed hamsters had significantly later offsets overall ($P < 0.001$), and a significant time×group interaction ($P < 0.001$) reflected the larger delay in the offsets observed for dim-exposed hamsters. Offsets occurred significantly later for dim-exposed hamsters at every time point from week 3 (Fig. 4B; $P < 0.05$). Consistent with these changes in onsets and offsets, activity duration was greater in dim-exposed hamsters ($P < 0.05$) and expanded more rapidly in that group ($P < 0.001$). At no

Fig. 1 Sample double-plotted actograms of hamsters entraining to LDLD7:5:7:5 with unsplit (**A**) or split (**B**, **C**) wheel-running rhythms. One hamster adopted the split pattern only after 2 weeks in this photoperiod (**B**). The time of transfer to constant conditions (*DD*) is noted on the left margin of each actogram. Light and dark rectangles above the actograms represent times of photophases and scotophases, respectively. Dark and dim scotophases are shaded *black* and *gray*, respectively. Wheel-running data are unfiltered and are scaled from 0 to 150 counts min^{-1}



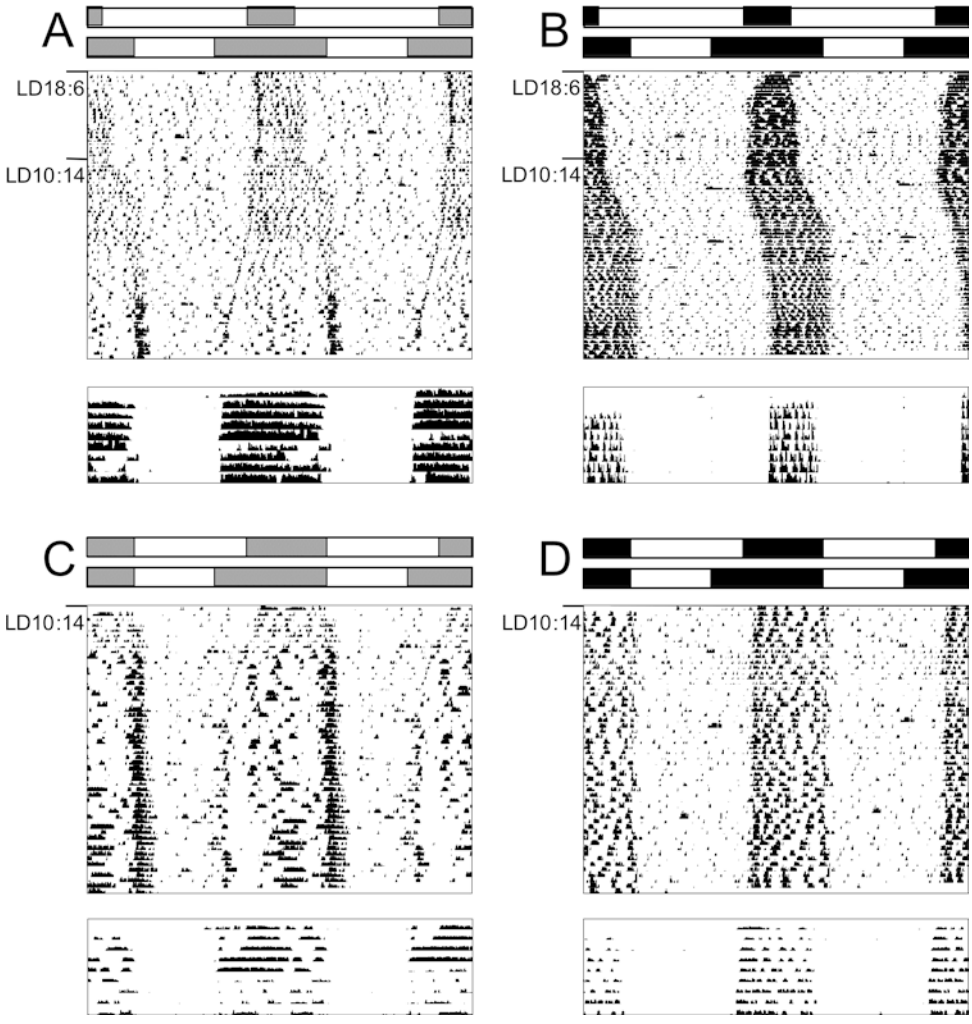


Fig. 3 Sample double-plotted actograms of hamsters transferred to LD 10:14 from LD 18:6 (**A**, **B**) or from LD 14:10 (**C**, **D**) where each scotophase was dimly illuminated (< 0.02 lx; **A**, **C**) or completely dark (**B**, **D**). Pre- and post-transfer photoperiods are depicted above the actograms and noted along the left margin of each actogram. For **C** and **D**, data collection began with exposure to LD 10:14, so the prior entraining photoperiod is depicted for reference only. Motion detector data are presented as percentile plots (larger actograms above). Wheel-running data are presented as raw activity counts (shorter actograms below) and are scaled from 0 to 100 counts min^{-1}. Other conventions as in Fig. 1

individual time point, however, did activity duration differ significantly between groups using the Bonferroni correction for multiple comparisons (Fig. 4B).

Gonad size and body weight

Four weeks following transfer from LD 18:6 to LD 10:14, ETV did not differ between dim- and dark-exposed hamsters ($P = 0.05$), but was significantly lower in the former group after 8 weeks in LD 10:14 ($P < 0.05$; Fig. 5A). Weight gain was significantly smaller for dim-exposed hamsters after 4 or 8 weeks in LD 10:14 ($P < 0.05$, $P < 0.001$, respectively; Fig. 5C).

Among hamsters transferred from LD 14:10 to LD 10:14, ETV at week 4 was significantly reduced among hamsters exposed to dim versus dark scotophases ($P < 0.01$) and this effect persisted through week 8 ($P < 0.01$; Fig. 5B). Dim versus dark-exposed hamsters did not show significant differences at week 4 in the change from baseline body weight, but by week 8, dim-exposed hamsters exhibited significant reductions compared to dark-exposed hamsters ($P < 0.05$; Fig. 5D).

Wheel-running activity

Among hamsters given running wheels in LD 10:14, activity duration was significantly longer for those exposed to dim compared to dark scotophases whether they were initially exposed to LD 18:6 (Fig. 6A; $P < 0.01$) or to LD 14:10 (Fig. 6B; $P < 0.05$). These group differences persisted when the dim illumination was discontinued over days 6–9 (Fig. 6A, B). One hamster pre-entrained to LD 14:10 failed to run in the wheel at a sufficient intensity to produce an interpretable activity record (activity counts were less than 200/day) and was thus excluded from this last analysis.

Nonresponders

In the final week of activity monitoring with motion detectors, only a single hamster transferred from LD 14:10 met the criterion for photo-nonresponsiveness of the circadian system. However, in this case activity duration calculated from wheel-running data was that of a responder. Among hamsters previously entrained to

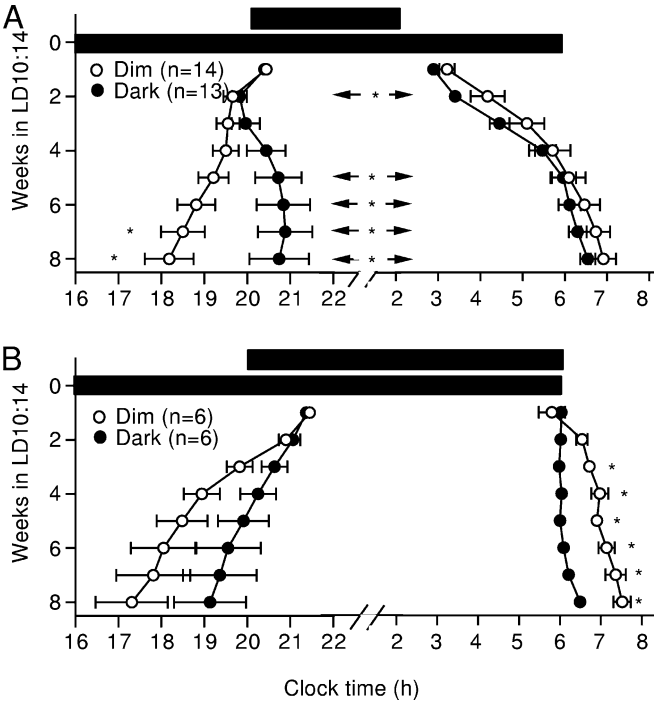


Fig. 4 Activity onsets and offsets of dim- versus dark-exposed hamsters following transfer to LD 10:14 from LD 18:6 (**A**), or from LD 14:10 (**B**). Data are mean ± SEM. To facilitate interpretation and comparison to actograms the ordinate has broken scaling that is centered on nocturnal activity. The timing of the scotophase immediately before and after transfer is indicated by the *two black rectangles* above the graphs. *Asterisks* indicate significant differences between dim and dark-exposed hamsters at individual time points ($P < 0.00625$ using Bonferroni correction for multiple comparisons). *Asterisks flanked by arrows* indicate significant group differences in activity duration, which is the interval between activity onset and offset

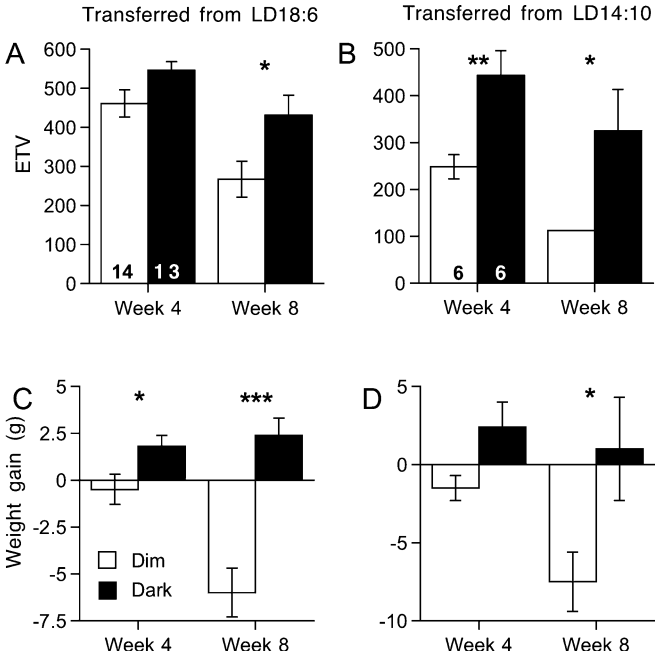


Fig. 5 Estimated testis volume (ETV) and weight gain of hamsters exposed to dim versus dark scotophases after 4 or 8 weeks in LD 10:14 following transfer from either LD 18:6 (**A**, **C**) or from LD 14:10 (**B**, **D**). Data are mean ± SEM. Sample size for both measures is indicated in the bars of the week 4 ETV graph. *Asterisks* indicate a significant difference between groups exposed to dim and dark scotophases (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$)

LD18:6, 5 hamsters exposed to dark scotophases and 1 in dim scotophases were considered nonresponders on the basis of motion detector data (χ^2; $P = 0.05$). As determined by the wheel-running criterion, significantly fewer nonresponders were observed with dim versus dark scotophases (χ^2; $P < 0.05$).

Atypical activity rhythms

Of the nine hamsters designated as having abnormal activity rhythms measured by motion detectors, six appeared weakly or completely arrhythmic. This characterization was confirmed by periodogram analysis of the final 10 days of the activity record. Rhythms of three other hamsters were atypically phased, exhibiting greater locomotor activity in the light than in the dark. Running wheel data corroborated the aberrant circadian organization in eight of these nine animals: In each, wheel-running was sporadically timed and at extremely low levels. During days 2–5 of wheel exposure, total wheel revolutions of these eight animals were 10.2% of the mean for the entire group. Finally, in no case did the significant effect of dim light on ETV or body weight hinge on the exclusion of these hamsters from the analysis.

Discussion

Dim nocturnal illumination influenced re-entrainment in two markedly different experimental paradigms—transfer from LD 14:10 to LDLD cycles and transfer from long to short photoperiods—that each induce changes in the phase relationship between discrete markers of circadian phase. These changes in phase relationships have been productively understood as reflecting changes in the phasing of multiple underlying circadian oscillators. Despite the shared functional outcome, the two paradigms were selected for the dissimilarities in the mechanisms by which these altered phase relationships are achieved.

Rhythm splitting in LDLD

The dim-light facilitation of rhythm splitting in a LDLD cycle mirrors that observed in Syrian hamsters with nocturnal illuminance reported as < 0.1 lx (Gorman et al. 2003). As the two studies employed identical lamps and caging, the value reported here (< 0.020 lx) represents a more precise light measurement rather than a lower-intensity light source. The mechanisms underlying

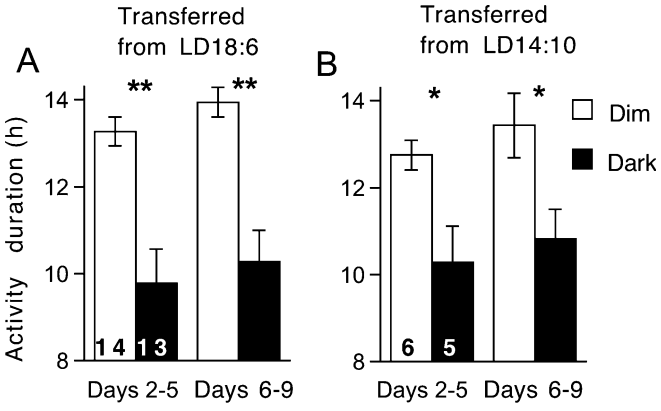


Fig. 6 Duration of wheel-running activity of hamsters in LD 10:14 with dim or dark scotophases following transfer from either LD 18:6 (**A**) or LD 14:10 (**B**). Data are mean ± SEM. During days 2–5 the prior lighting conditions (dim versus dark) were maintained, but all hamsters were exposed to completely dark scotophases during days 6–9. Sample size is indicated at the base of each bar. One dark-exposed hamster transferred from LD 14:10 was excluded because too few wheel revolutions were recorded for determination of activity duration (α). Conventions as in Fig. 5. *Asterisks* indicate differences between dim- and dark-exposed groups (*$P < 0.05$; **$P < 0.01$)

LDLD-entrained splitting have been addressed to date only in Syrian hamsters. In that species, repeated phase-shifting of component oscillators by novelty-induced wheel running may be sufficient to induce the split circadian entrainment pattern (Mrosovsky and Janik 1993; Gorman and Lee 2001; Evans and Gorman 2002). In other experimental contexts, compression of subjective night by long photophases and/or direct, light-induced resetting of pacemaker phase may contribute to rhythm splitting (Gorman 2001; Gorman et al. 2003). Thus, splitting arises in association with phase-resetting by induced activity and/or light. Its unequivocal role in rhythm splitting notwithstanding (Gorman et al. 2003), dim light in Syrian hamsters appears not to substantially alter phase-shifts elicited by activity or light stimuli presented to unsplit animals free-running in constant conditions. Nor is 1 h of dim light sufficient to induce a phase shift in animals free-running in complete darkness (M.R. Gorman and J.A. Elliott, unpublished observations). Although comparable studies in Siberian hamsters are not available, the similar responses to LDLD 7:5:7:5 (Gorman and Elliott 2003) suggest that splitting likely depends on homologous mechanisms in these two species.

Re-entrainment to winter daylengths

The circadian mechanisms underlying the photoperiodic response to short daylengths are much better characterized than those involved in LDLD-induced splitting. According to the two-oscillator model, the duration of subjective night reflects the phase angle of two coupled oscillators that are principally entrained by morning and evening light/dark (L/D) transitions (Pittendrigh 1974; Pittendrigh and Daan 1976; Daan and Berde 1978). Phase-delaying and phase-advancing actions of evening and morning light, respectively, induce compression of α in long summer photoperiods. If a hamster entrained to long daylengths is exposed to constant darkness, subjective night gradually lengthens as the E and M oscillators drift earlier and later, respectively under the influence of different intrinsic free-running periods and/or oscillator interactions (Elliott and Tamarkin 1994; Gorman et al. 1997). The same gradual expansion of α can be observed following transfer from long to short photoperiods if the L/D and D/L transitions are adjusted symmetrically. This similarity suggests that this re-entrainment to short daylengths chiefly reflects changes intrinsic to the pacemaker rather than direct resetting actions of light pulses.

In experiment 2, the transfer from LD 14:10 to LD 10:14 was accomplished via an advance of the L/D transition exclusively. Under these conditions, the morning phase marker generally remains entrained to the unchanging D/L transition, and α expansion depends on gradual advances of the evening phase marker (Illnerova et al. 1986; Illnerova 1991). The timing of the LD 14:10 to LD 10:14 photoperiods was chosen to distinguish an effect of dim light on oscillator interactions from one on free-running period (τ) of the pacemaker. According to Aschoff's rule, the free-running period measured by activity onsets lengthens with light intensity (Aschoff 1960), and this effect depends on the action of light during subjective night (Ferraro and McCormack 1984; Ferraro 1990). If τ is lengthened by nocturnal exposure to dim illumination, then activity onsets should advance more slowly than they do with dark scotophases or, alternatively, may not advance at all. Because dim light accelerated advances of this circadian marker, some other action of dim light is implied. Unexpectedly, dim illumination also promoted delays in activity offset, an effect that would be consistent with a longer offset τ among dim-exposed hamsters.

The same advancing effect of dim light on activity onsets was noted following symmetric transfers from LD 18:6 to LD 10:14, whereas activity offsets were unaffected. Application of the short-day re-entrainment model described above, however, is complicated by the use of very long daylengths such as LD 18:6, which induces arrhythmia in a sizable fraction of hamsters and photo-nonresponsiveness in others (Gorman and Zucker 1997; Prendergast and Freeman 1999). This latter fraction of hamsters transferred from long to short daylengths fails to adopt a winter phenotype because the circadian system never adopts a long subjective night (Puchalski and Lynch 1988). While artificial selection experiments demonstrate a genetic basis for this trait (Kliman and Lynch 1992; Freeman and Goldman 1997), the expression of photo-nonresponsiveness depends on prior exposure to very long daylengths (Gorman and Zucker 1997; Goldman and Goldman 2003). Once the circadian system of the predisposed hamster entrains to very long daylengths, α will not expand in constant

darkness. At a formal level of analysis, this has been suggested to reflect altered τs of the E and M oscillators, or to an altered coupling dynamic between them. As in previous studies (Gorman and Zucker 1997; Prendergast and Freeman 1999), a sizeable fraction (26%) of the total sample exposed to LD 18:6 was arrhythmic or abnormally entrained and another 17% failed to lengthen α beyond 8 h. Following LD 14:10, only 7% were arrhythmic and 7% nonresponsive. The presence of dim nocturnal illumination concurrent with exposure to LD 18:6 or afterwards essentially eliminated photononresponsiveness but offered no protection from the aberrant circadian rhythmicity or arrhythmicity induced by these very long photoperiods.

As described above for the expansion of α following transfer from LD 14:10, classical effects of light cannot explain the reduced incidence of nonresponsiveness in dim light. If a longer τ is the causal basis of nonresponsiveness as discussed in some reports (Freeman and Goldman 1997; Gorman et al. 1997; Gorman and Zucker 1997; Prendergast and Freeman 1999), then dim light would be expected to increase rather than decrease the incidence of this phenotype. The absence of running wheels in experiment 2, moreover, eliminates the possibility that dim light alters wheel-activity-dependent changes in pacemaker function (Freeman and Goldman 1997). Instead, the effects of dim light in both experiments may be parsimoniously explained by an effect on oscillator coupling. Until interactions between oscillators are better understood, however, it will not be possible to state precisely how dim light alters them. In LDLD, dim-light-facilitated splitting represents adoption of a novel phase relationship between circadian oscillators yet to be identified. The entrainment pattern persists indefinitely under LDLD but is rapidly lost in constant conditions, revealing intrinsic instability of this circadian configuration. In contrast, following transfer from long to short daylengths, dim light accelerates adoption of a phase angle between component oscillators that is preferred in short daylengths (and DD) and characterized by maximally expanded α. Dim nocturnal illumination may simply decrease the stability of the compressed α state that is induced by moderate and very long photoperiods. An effect of dim illumination that more explicitly implicates oscillator coupling was apparent in an activity record of a single tree shrew maintained in constant 2.8 lx. Two distinct activity components were apparent, one free-running with a consistent τ and the second exhibiting relative coordination. Reduction of light intensity to 0.1 lx clearly altered the coupling as it induced the two components to adopt a stable phase angle (Meijer et al. 1990).

Few studies to date have systematically addressed pacemaker function under low levels of nocturnal illumination. In bats entrained to LD 12:12, nocturnal illumination from 1×10^{-2} to 1×10^{-6} lx affected the waveform of the entrained rhythm in general activity (Erkert et al. 1976), but that study did not differentiate effects on pacemaker organization from downstream effects on overt behavior. Besides altering entrainment of the circadian pacemaker, nighttime illumination is known to acutely affect activity levels (Erkert 1976; Erkert and Grober 1986; Kavanau 1967; Mrosovsky 1999). In the present context, a masking effect of dim light on locomotor activity (e.g., causing increased levels) fails to account for the enhanced melatonin-dependent changes in body weight and gonadal condition that accompanied changes in the activity rhythm. A masking interpretation is further discounted by the finding that, when all animals were placed in LD 10:14 with completely dark scotophases, those previously exposed to dim light continued to exhibit longer activity durations.

Recent studies have identified melanopsin-containing retinal ganglion cells as principal transducers of light information to the SCN (Berson 2003). In mice, targeted mutations of the melanopsin gene markedly diminished the effects of light on phase-resetting, pacemaker period, and pupillary constriction (Panda et al. 2002; Ruby et al. 2002). The intensity of light required for activation of these cells, and for phase-resetting actions of light, is an order of magnitude or more greater than that shown here to alter circadian re-entrainment (Berson 2003). This observation supports the conclusion that dim illumination alters circadian systems through mechanisms distinct from those involved in photic phase-resetting and parametric modulation of pacemaker period.

Coupling remains one of the most imprecisely defined and experimentally elusive concepts of circadian rhythms research. Further support for the hypothesis that dim nocturnal illumination alters oscillator coupling will depend on convergent evidence from multiple experimental paradigms. The identification of factors that modulate this process will help define the nature of oscillator interactions and will facilitate clarification of underlying physiology.

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