

Research report

# Circadian entrainment and phase resetting differ markedly under dimly illuminated versus completely dark nights

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## Abstract

An endogenous circadian pacemaker uses photic input to synchronize mammalian physiological and behavioral rhythms to the 24 h day. Sunlight during dusk and dawn is thought to entrain the pacemaker of nocturnal rodents, whereas moonlight and starlight are presumed to exert little influence. We show that, to the contrary, dim illumination (<0.005 lux), similar in intensity to starlight and dim moonlight, markedly alters entrainment of hamster activity rhythms. Under 24 h light:dark:light:dark cycles (LDLD), for example, activity rhythms can disassociate, or split, into two distinct components, and the incidence of split entrainment is increased when daily scotophases are dimly lit rather than completely dark. The three present studies examine whether dim illumination promotes LDLD-induced splitting (1) by increasing nonphotic feedback during novelty-induced activity bouts, (2) by potentiating nonphotic and/or photic resetting, or (3) by influencing phase jumping responses under skeleton photoperiods simulating increases in day length. Experiment 1 illustrates that dim-exposed animals display split rhythms, while animals without dim light do not, despite equivalent activity levels. In Experiments 2 and 3, dim illumination potentiated both nonphotic and photic resetting, and the specific nature of these interactions suggests mechanisms through which dim illumination may alter entrainment under LDLD. Dim light likely promotes LDLD-induced splitting by facilitating both nonphotic resetting and bright light-induced phase jumping in animals entrained to short nights. The actions of dim illumination may be distinct from canonical responses to bright light, and potentially influence the interactions between oscillators comprising the circadian pacemaker.

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## 1. Introduction

Day and night are often simulated in the laboratory by 24 h lighting regimes alternating between moderate indoor light levels and complete darkness. Such light:dark cycles are sufficient to entrain activity rhythms in most mammals, including the rodents commonly used to characterize the circadian system. In the wild, however, nocturnal rodents are

rarely active in complete darkness and instead emerge from darkened burrows at night to navigate a landscape dimly lit by the moon and stars, which can provide illumination as high as 0.04 and 0.3 lux at the quarter and full moons, respectively [3,6,42]. Such dim illumination is commonly thought to have little influence on the circadian system since light at this intensity does not appear to produce phase shifts or suppress melatonin secretion—two hallmark circadian responses to light exposure during subjective night [6,7,30,31].

Challenging this view, we have shown that nocturnal illumination comparable in intensity to that of dim moonlight markedly alters entrainment of hamster activity rhythms across several distinct paradigms. In Siberian hamsters transferred from long day to short day photoperiods, the duration of nocturnal activity lengthens more rapidly under dimly lit

*Abbreviations:* LDLD, light:dark:light:dark cycle; PIR, passive infrared;  $\tau$ , free-running period;  $\alpha$ , nocturnal activity duration;  $\psi_{LD}$ , phase angle of entrainment to the light to dark transition

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nights than completely dark nights, and other photoperiodic responses are likewise accelerated [13]. Further, dim illumination markedly alters the entrainment of Syrian hamsters held under more exotic conditions such as non 24 h lighting schedules (i.e. T cycles) [17] and 24 h light:dark:light:dark (LDLD) cycles [13,15]. Within this latter paradigm, some animals entrain in a conventional, unimodal manner with wheel running activity restricted to one scotophase of the LDLD cycle, whereas other individuals regularly divide activity between the two daily scotophases (i.e. they exhibit “split” rhythms) [11,12,14,15,18,19]. Dim illumination during the two scotophases (i.e. dim “scotopic” illumination), rather than complete darkness, more than triples the incidence of these split rhythms [13,15]. Entrainment under each paradigm has been interpreted in the context of multi-oscillator models of the circadian pacemaker, first articulated by Pittendrigh and Daan [35]. In each case, circadian re-entrainment is thought to involve changes in the phase relations between two or more coupled, or interacting, pacemakers. As scotopic illumination is highly effective in each of these paradigms, we have proposed that it alters the interactions between putative oscillators [13,15]. The present studies examine for the first time the role of dim illumination in LDLD-induced splitting in terms of basic entrainment processes.

Circadian phase can be reset in a time-gated fashion by both nonphotic and photic stimuli [20,26], and each type of zeitgeber has been posited to contribute to the induction of split rhythms under LDLD cycles [12,15,18]. For example, when “novel wheel running” (NWR) is repeatedly scheduled during subjective day, animals that engage in robust NWR later exhibit split rhythms, whereas less active “slug-gards” do not [11,18,19,28,40]. In other experiments using LDLD, split rhythms appear to be triggered by activity induced by transfer to a wheel running cage or by a cage change [15]. These results are consistent with a hypothesis that nonphotic phase shifts of distinct oscillator populations contribute to the emergence of split rhythms under LDLD [12,15,18].

Bright light is likewise implicated in splitting under LDLD since split rhythms are not sustained under constant darkness (i.e. activity components rapidly rejoin) [14,18]. Additionally, an inductive role for the bright light of LDLD cycles is suggested by the spontaneous emergence of a second activity bout when the duration of the scotophase entraining nocturnal activity is sufficiently reduced [12]. The emergence of these LDLD-induced split rhythms bears some resemblance to the re-entrainment that occurs under a “skeleton” photoperiod when the two light pulses simulate increasing day lengths. Under these conditions a “phase jump” occurs, where activity abruptly crosses one of the entraining light pulses and realigns into the longer scotophase previously reflecting subjective day [34,41]. Both phase jumps and LDLD-induced split rhythms emerge after bright light compresses the duration of subjective night, thereby challenging circadian entrainment. Beyond a “minimum tolerable night” [34,41], the

resulting expression of a phase jump, or a split rhythm, may be dependant on the duration of the alternative scotophase [15].

Published photic fluence response curves in this species suggest that the intensity of dim illumination used in our prior studies would not produce phase shifts on its own [7,30,31]. We noted in an earlier study that male Syrian hamsters with dimly lit scotophases displayed higher wheel running levels than animals with completely dark scotophases [15]. Dim light could promote LDLD-induced splitting by increasing the effective dose of the nonphotic stimulus during novelty-induced activity bouts under LDLD. Alternatively, dim illumination may potentiate phase resetting responsiveness to nonphotic cues and/or bright light. Finally, as LDLD-induced split rhythms may reflect changes in the phase relations of coupled oscillators [15], scotopic illumination could exert its influence by altering circadian coupling. Lacking a direct assay of circadian coupling [25,39], we may best infer whether dim light operates at this level through convergent analysis of circadian behavior under various paradigms where changes in behavior are thought to depend on adjustments in the phase relations of coupled oscillators [33,35].

Each of the three following experiments characterizes the influence of dim illumination on a factor potentially contributing to the emergence of LDLD-induced split rhythms. Specifically, we assessed whether dim illumination operates by modulating (1) novelty-induced activity levels, (2) nonphotic and bright photic phase resetting and (3) re-entrainment under skeleton photoperiods. Experiment 1 replicates and extends our previous report, demonstrating that LDLD-induced splitting is increased with dimly illuminated scotophases but not by augmented wheel running intensity in the absence of dim light. Incorporating manipulations designed to mimic the nonphotic and bright photic stimuli under LDLD, Experiment 2 examined whether phase resetting is differentially influenced by dimly lit versus dark free-running conditions. Lastly, Experiment 3 investigated whether scotopic illumination affects the emergence of phase jumps elicited by skeleton photoperiods simulating increases in day length. These two latter experiments demonstrate that phase resetting and phase jumping are altered by dim light in a manner that may involve changes in the interactions between coupled oscillators.

## 2. Materials and methods

### 2.1. General methods

#### 2.1.1. Breeding and initial husbandry conditions

For each of the following experiments, female Syrian hamsters (*Mesocricetus auratus*) were bred from stock originally purchased from Harlan (HsdHan:AURA, Indianapolis, IN) and reared within our laboratory under a 14 h light:10 h dark photocycle (LD 14:10, lights on: 0300 PST, lights off: 1700 PST). During this time, 40 W fluorescent bulbs provided photophase il-

lumination of 100–300 lux at cage lid, with complete darkness during the scotophase (i.e. no computer lights or other extraneous light sources). Prior to use in experiments, animals were group-housed without running wheels inside polypropylene cages (48 cm × 27 cm × 20 cm) located on open racks, with room temperature maintained at  $22 \pm 2^\circ\text{C}$ . Food (Purina Rodent Chow #5001, St Louis, MO) and tap water were available ad libitum. Hamsters (age 10–12 weeks) were transferred to individual light-tight housing units for each of the following experiments, which were conducted in compliance with all the rules and regulations of the Institutional Animal Care and Use Committee, University of California, San Diego.

### 2.1.2. Scotopic illumination

Scotopic illumination was provided by green light-emitting diodes (LEDs; Arcoelectric, Thousand Palms, CA) mounted in the back wall of each individual housing unit. These LEDs have a peak transmission wavelength of 560 nm with a one half bandwidth of 23 nm as measured by an Ocean Optics PS1000 spectrometer (Dunedin, FL). While this scotopic illumination has been conservatively reported as  $<0.1$  lux [13,15], more precise measurements conducted with an IL1705 Radiometer system (International Light, Newburyport, MA) revealed that the dim light intensity used in the current and previous studies is even lower than previously documented. As measured at hamster eye level in the brightest region of the cage floor, scotopic illumination used currently was  $4.2 \times 10^{-3}$  lux and  $7.9 \times 10^{-6}$   $\mu\text{W}/\text{cm}^2$ , equivalent to  $2.23 \times 10^9$  photons/ $\text{cm}^2$  s.

### 2.1.3. Rhythm monitoring and analyses

Activity rhythms were primarily monitored via home cage running wheels (diameter = 17 cm) located within polypropylene cages (27 cm × 20 cm × 15 cm). Entrainment was monitored in wheel-naïve animals (Experiment 2) via passive infrared (PIR) motion detectors (Coral Plus, Visonic, Bloomfield, CT) positioned ~32 cm above the cage floor of cylindrical polyethylene cages (26 cm diameter). Half revolutions of home cage wheels or movement under PIR sensors triggered closures of a relay, which were collected and compiled into 6 min bins by DataQuest III or Vital View software (Mini-Mitter, Bend, OR).

Actograms were prepared and analyzed with Clocklab software (Actimetrics, Evanston, IL). As in a previous report [15], the scotophase reflecting the phase of the animals' subjective night at the beginning of the experiment is referred to as the "nighttime" scotophase, while the scotophase added during the experiment is designated the "daytime" scotophase. Similarly, the photophases occurring before and after the nighttime scotophase are labeled the "evening" and "morning" photophase, respectively. These conventions are illustrated in Figs. 1 and 2.

### 2.1.4. Statistical analyses

Categorical data were analyzed using contingency statistics (Pearson's  $\chi^2$ ). Continuously varying activity and entrainment measures were assessed primarily using parametric statistics. When significant heterogeneity of variance was detected between groups, Kuskall-Wallis nonparametric tests were performed, and these values are reported instead. Statistical tests were conducted with JMP software (SAS Institute, Cary, NC) and values in text and illustrations are expressed as mean  $\pm$  S.E.M.

## 2.2. Experiment 1

### 2.2.1. Procedures

Split rhythms were generated in a manner similar to that described previously [15]. Seven hours after lights on, wheel-naïve animals were transferred to individual cages equipped with running wheels. Transfer corresponded to the beginning of the daytime scotophase of the new LDLD cycle (LDLD 7:5:7:5; lights off: 1000; lights on: 1500, lights off: 2200, lights on: 0300 PST). Thereafter, photophase light intensity was 50–75 lux and scotophase illumination depended on group assignment, as detailed below. A cage change was performed two weeks after transfer. During the first 90 min of the daytime scotophase, animals and their wheels were transferred to cages with fresh bedding, water and food under the direction of dim red head lamps ( $<1$  lux for  $<5$  min/animal).

At the time of the initial transfer, hamsters were randomly assigned to one of three groups that differed in the intensity of scotophase illumination and the type of wheel provided. One group received scotopic illumination and cages equipped with standard (i.e. unmodified) wheels (DIM-Std Wheel,  $n=7$ ). For the two remaining groups, scotophases were completely dark, and animals received cages equipped with either standard wheels (DARK-Std Wheel,  $n=7$ ) or modified wheels (DARK-Mod Wheel,  $n=8$ ), where the metal rungs were wrapped with a plastic guard to increase wheel-running coordination (c.f. [29]).

### 2.2.2. Analyses

For analytic purposes, this experiment was divided into two 2-week intervals, beginning with the initial transfer and cage change, respectively. Group differences in split rhythm incidence and novelty-induced activity were analyzed separately for each interval. Activity rhythms were categorized as split if animals expressed wheel running bouts longer than 30 min during both daily scotophases for at least five consecutive days. Consistent with previous experiments [14,15], there was no ambiguity in classifying animals as split or unsplit. Additionally, wheel running counts across the first three days of each interval were summed for individual animals in hourly bins. Group differences in total wheel revolutions were assessed for each scotophase and photophase across the first three days of each interval. NWR was operationally defined as total wheel revolutions expressed during the 5 h daytime scotophase coincident with the initial transfer or cage change.

## 2.3. Experiment 2

Experiment 2 examined whether dim light alters phase resetting induced by the nonphotic and photic stimuli associated with the emergence of split rhythms in LDLD, using procedures specifically designed to mimic conditions of Experiment 1. Dim light may influence the sensitivity to novelty-induced activity bouts and thereby potentiate nonphotic phase resetting theorized to operate on the first day of each interval under LDLD. Animals splitting in the two different intervals of Experiment 1 had different photoperiodic histories. Those animals splitting in Interval 1 had been just previously entrained to LD 14:10, while animals splitting in Interval 2 were previously entrained to LDLD 7:5:7:5, which is technically a skeleton photoperiod of LD 19:5. Thus, LD 14:10 and LD 19:5 were used presently to simulate differences in entrainment prior to Intervals 1 and 2, respectively. Lastly, after the initial transfer to LDLD and intense NWR, animals during Interval 1 receive bright light

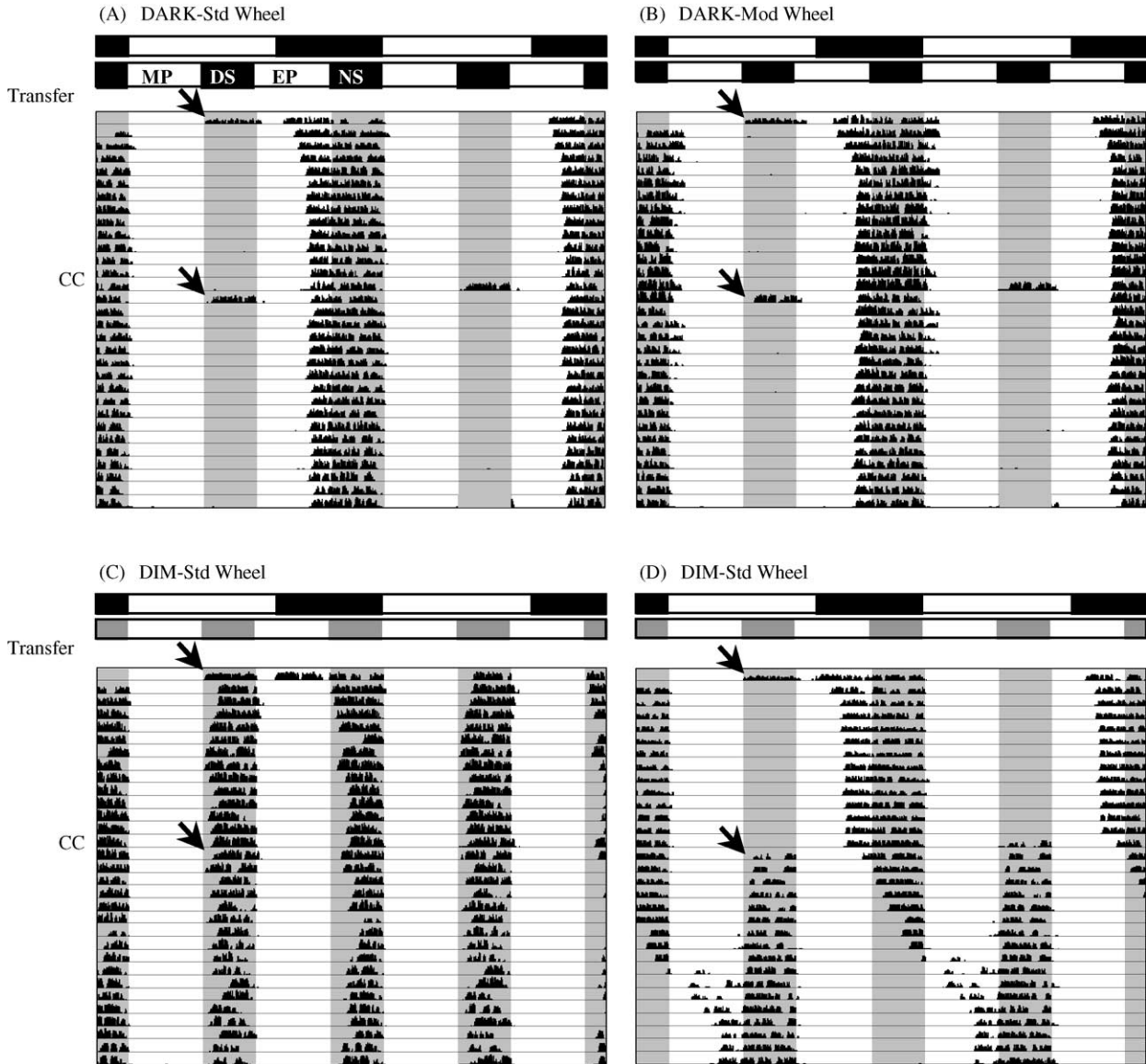


Fig. 1. Representative double-plotted wheel-running actograms depicting unsplit and split rhythms exhibited by hamsters during Experiment 1. Light:dark bars above each actogram represent photoperiods in effect before (top bar) and during the experiment (bottom bar; also internal shading). White rectangles represent photophases, and shaded black bars represent DIM and DARK scotophases, respectively. MP = morning photophase; DS = daytime scotophase; EP = evening photophase; NS = nighttime scotophase. First and second arrows indicate the time of transfer to wheel running cages and cage change, respectively. Actograms are scaled 0–150 counts/min.

exposure during early subjective night (e.g. former ZT 12–ZT 17). This experiment also assessed whether dim illumination influences phase resetting induced by this compound stimulus (i.e. NWR plus bright light).

2.3.1. Procedures

Animals were individually housed without running wheels in cylindrical polyethylene cages (35 cm height × 26 cm diameter). For 28 days, animals were entrained to either LD 14:10 (lights on: 0300 PST, lights off: 1700 PST) or LD 19:5 (lights on: 0300 lights off: 2200 PST), during which activity rhythms were monitored with PIR. Photophase and scotophase intensity during entrainment was ~100 and 0 lux, respectively. Midway through this entrainment period, cages were cleaned during the photophase and a

handful of soiled bedding was retained in an effort to reduce novelty-induced activity. On one day only, the lights-off transition (zeitgeber time = ZT 12) was advanced by 5 h in order to determine whether activity onset was negatively masked by light during entrainment to LD 14:10 and LD 19:5.

As indicated above, phase shifting conditions were designed to mimic the nonphotic and photic stimulation used during the LDLD-induced splitting paradigm. As illustrated in Fig. 3, phase shifts were studied under a modified Aschoff Type II design [1], where release into constant conditions coincides with the application of phase shifting manipulations. Seven hours after lights on, animals were transferred from LD 14:10 (transfer at ZT 5) or LD 19:5 (transfer at ZT 0) to cages with modified running wheels (see Experiment 1). Animals from each photoperiod were transferred to wheel running



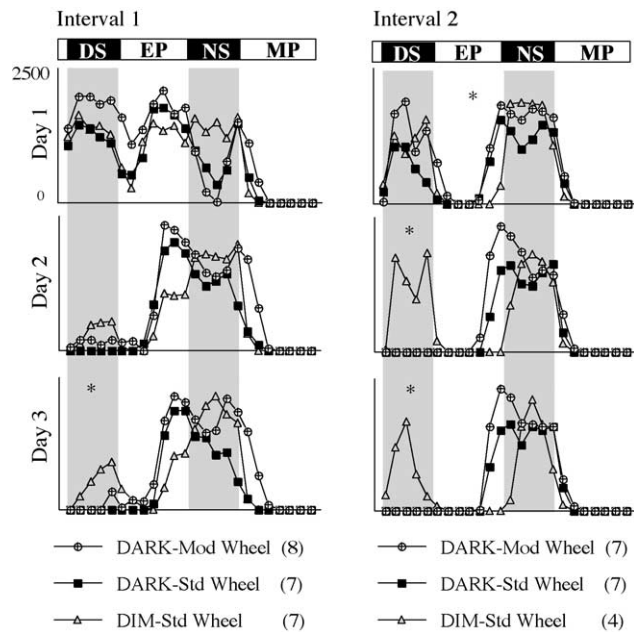


Fig. 2. Mean hourly counts for the first 3 days of each interval in Experiment 1. For figure clarity, standard errors are not shown. Asterisks signify phases of the photocycle (i.e. EP, DS) where the DIM-Std Wheel group displayed activity levels significantly different from the two DARK groups ( $p < 0.05$ ). The number in parentheses is the number of animals per group. Animals that split during Interval 1 were excluded for Interval 2. Abbreviations as in Fig. 1.

cages with complete darkness (LD 14:10-DARK;  $n = 7$ ; LD 19:5-DARK;  $n = 8$ ) or with dim illumination (LD 14:10-DIM;  $n = 8$ ; LD 19:5-DIM;  $n = 8$ ). No attempt was made to control for the intensity or duration of subsequent wheel running. To determine whether dim light influenced the response to the compound stimulus, two additional groups of LD 14:10 animals received a 7 h light pulse (50–75 lux) after 5 h of NWR in complete darkness (LD 14:10-DARK + L,  $n = 8$ ) or in dim illumination (LD 14:10-DIM + L,  $n = 8$ ). After phase shifting manipulations were complete, animals remained in constant conditions for two weeks to calculate phase shift magnitude and free running period length ( $\tau$ ).

### 2.3.2. Analyses

Using PIR actograms in the Clocklab percentile format, activity onset and offset were determined for each day over the last two weeks under entrained conditions (Week 3 and Week 4), and a regression line was fit to each set of seven points. The average length of activity ( $\alpha$ ) for each week was derived from the difference between average onset and offset. Average activity onset is expressed as the phase angle of entrainment to the light to dark transition ( $\psi_{LD}$ ), which is the time difference between the entraining and behavioral event. PIR actograms were visually inspected for activity onset on the day of the dark probe by noting the first 6 min bin after lights off when activity exceeded two counts and was sustained for at least 5 of 8 subsequent bins.

A phase shift was determined for each animal by the displacement between the average activity onset during Week 4 and the time of activity onset predicted for the day of transfer by a regression line fit to visually-identified wheel running onsets (7 post-pulse days were used, excluding the first four to allow for transients).

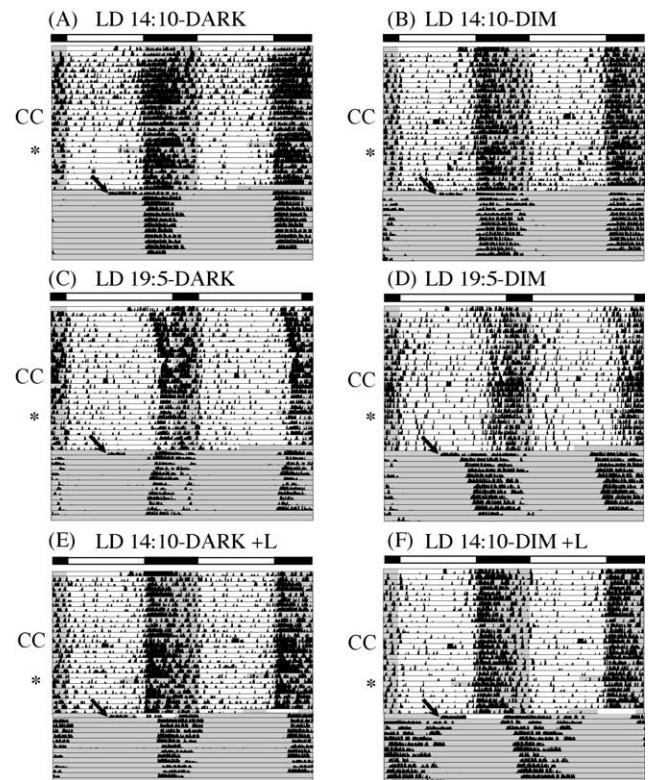


Fig. 3. Representative double-plotted actograms depicting entrained and free-running activity rhythms exhibited during Experiment 2. White and black bars above each actogram represent the photocycle in effect while activity was monitored with passive infrared motion detectors (PIR). The change to internal shading marks the day of transfer to wheel running cages (for convenience, shading begins at midnight) and the arrow marks the time of transfer. Entrained PIR rhythms are in Clocklab's percentile format, whereas free-running wheel running rhythms are scaled from 0 to 150 counts. The day of the cage change (CC) and the dark probe (asterisk) are indicated. For the day of the dark probe, the light to dark transition was advanced by 5 h, as represented within each actogram. White boxes on the day of transfer in (E) and (F) represent 7 h light pulses.

Pre- and post-pulse activity rhythms were monitored via different methods (PIR or wheels), which precludes a precise specification of the absolute size of phase shifts [2]. Phase shifts were determined identically for every group, however, so that DIM versus DARK differences could be assessed. Lastly, the slope of the post-pulse regression line was used to calculate  $\tau$  and this value was compared between groups free-running in constant dim and dark conditions.

### 2.4. Experiment 3

Ultra long photoperiods (>16–18 h) challenge circadian entrainment in nocturnal rodents, resulting in the expression of a phase jump if animals are held under skeleton photoperiods simulating increases in day length [34,38,41]. A similar mechanism may contribute to the temporal disassociation of component oscillators under LDLD [15]. Experiment 3 was designed to determine whether dim illumination would influence the timing and pattern of phase jumps under skeleton photoperiods. Moreover, this paradigm assesses whether dim light influences photic entrainment when novelty-induced activity is minimized.

2.4.1. Procedures

Hamsters were held under a series of skeleton photoperiods, where the interval between entraining light pulses was systematically reduced (see Fig. 6). Under this series of photocycles, scotophases were marked by either complete darkness (DARK,  $n = 16$ ) or dim illumination (DIM,  $n = 16$ ). On the first day of the experiment, hamsters were transferred from LD 14:10 to running wheel cages identical to those used in Experiment 2. Although this transfer occurred during subjective day, the house lights remained on after transfer, and a new light:dark cycle was immediately instated by symmetrically reducing the following scotophase by 3 h (LD 17:7; lights on: 0130 lights out: 1830 PST). LD 17:7 was replaced one week later by an equivalent skeleton photoperiod with two 3 h light pulses (LDLD 3:11:3:7; lights on: 0130, lights off: 0430, lights on: 1530, lights off: 1830 PST). At weekly intervals thereafter, the nighttime scotophase was symmetrically reduced by 30 min. The duration of the daytime scotophase increased equivalently. Cage changes were performed during the evening photophase and a handful of soiled bedding was retained in an effort to reduce novelty-induced activity.

2.4.2. Analyses

Phase jumps were identified for individual animals by visually identifying the first day when a wheel running bout at least 18 min long was phased within the daytime scotophase and then repeated on at least three of the four subsequent cycles. The length of the nighttime scotophase at the time of the phase jump and the number of cycles preceding the phase jump were recorded for each animal and used to compare DARK and DIM groups. Once a phase jump was initiated, we noted the number of cycles that elapsed before activity was completely realigned into the daytime scotophase.

24 h histograms were produced for each hamster by averaging wheel revolutions within each 6 min bin across the seven days of each photocycle used in this experiment. Activity onset was defined as the first 6 min bin surpassing the daily mean that was followed by two bins likewise exceeding this threshold. Activity offset was defined as the last time point below the daily mean that was immediately preceded by two bins above threshold.  $\alpha$  was calculated as before, and  $\psi_{L/D}$  was derived as the difference between lights off for the nighttime scotophase and activity onset. These measures were then used to compare entrainment of DIM and DARK animals during the first four weeks of the experiment (i.e. before a large number of animals expressed phase jumps). Additionally,  $\alpha$  was determined for individual animals during the week before a phase jump and during the final week of the experiment.

3. Results

3.1. Experiment 1

3.1.1. Emergence of split rhythms

A variety of unsplit and split activity patterns was observed (Fig. 1). Hamsters that restricted activity to the nighttime scotophase were classified as unsplit (Fig. 1A and B), while hamsters that displayed activity in each of the two daily scotophases were classified as split, regardless of whether the split rhythm developed during Interval 1 (Fig. 1C) or Interval 2 (Fig. 1D). As illustrated in Fig. 2, split rhythms emerged in two different patterns: either developing gradually, with daytime scotophase activity accruing on subsequent days (Interval 1), or appearing abruptly, with a robust activity bout appearing in the daytime scotophase (Interval 2). Split rhythms also varied in their stability: either remaining split over both intervals (Fig. 1C) or consolidating activity into the daytime scotophase (Fig. 1D). The former pattern was generally characteristic of split rhythms developing after the initial transfer, while the latter pattern was observed in all animals that split after the cage change.

3.1.2. Splitting incidence

The incidence of splitting depended on scotopic illumination (Table 1). In Interval 1, DIM-Std Wheel animals tended to exhibit split rhythms more frequently than animals in either DARK group ( $\chi^2(2) = 4.59, p = 0.08$ ). During Interval 2, DIM-Std Wheel animals exhibited a significantly higher incidence of splitting than either DARK group, even when previously split animals were excluded from the analysis ( $\chi^2(2) = 14.49, p < 0.001$ ). Considering splitting incidence over both intervals, all DIM-Std Wheel animals exhibited split rhythms, while all but one DARK animal had unsplit rhythms ( $\chi^2(2) = 18.22, p < 0.001$ ).

3.1.3. Wheel running in LDLD

Group differences in splitting occurred despite the fact that animals within DIM-Std Wheel and DARK-Mod Wheel groups exhibited comparable NWR levels (Table 1; Fig. 2). Dim light did not significantly increase NWR during Interval 1, but DARK-Mod Wheel animals ran at significantly

Table 1  
Splitting incidence and novel wheel running (NWR<sup>a</sup>) during Experiment 1

	Interval 1			Interval 2			Intervals 1 and 2	
	Split	Unsplit	NWR	Split	Unsplit	NWR	Split	Unsplit
DIM-Std Wheel	3	4	7.4 ± 0.8	4	0	5.3 ± 1.3	7	0
DARK-Mod Wheel	1	7	8.8 ± 0.6	0	7	6.0 ± 1.0	1	7
DARK-Std Wheel	0	7	6.2 ± 0.6 <sup>b</sup>	0	7	3.5 ± 1.0	0	7
	0.1 > p > 0.05			p < 0.05			p < 0.0001	

<sup>a</sup> Wheel running revolutions (in thousands) during the 5 h afternoon scotophase coincident with transfer or cage change.

<sup>b</sup> Running levels significantly lower than DARK-Mod Wheel ( $p < 0.05$ ).

higher levels than DARK-Std Wheel animals ( $F(2, 19) = 4.64$ ,  $p < 0.05$ ). Similarly, during Interval 2, scotopic illumination did not influence NWR levels of theretofore unsplit animals ( $F(2, 15) = 1.68$ ,  $p > 0.2$ ).

For each interval, all three groups displayed a transient decrease in wheel running after the NWR displayed during the daytime scotophase (Fig. 2). Activity levels within the subsequent photophase and scotophase did not differ between groups on the first day of Interval 1. Wheel running levels across the first day of Interval 2 were similar, except that the evening photophase activity was reduced, and DIM-Std Wheel animals were less active than either DARK group ( $p < 0.05$ ). Over the course of the subsequent two days in Intervals 1 and 2, developing split rhythms were evident for the DIM-Std Wheel animals but not for DARK animals.

### 3.2. Experiment 2

#### 3.2.1. Entrainment to LD 14:10 and LD 19:5

As expected, hamsters displayed photoperiod-dependent differences in entrainment prior to the phase shifting manipulations (Fig. 3). While under their respective photoperiods, LD 14:10 animals expressed longer active phases than LD 19:5 animals (e.g. Week 4  $\alpha$ : LD 14:10 =  $9.88 \pm 0.16$  h, LD 19:5 =  $8.2 \pm 0.22$  h; Kuskall-Wallis Test;  $p < 0.001$ ) and also initiated activity closer to the light to dark transition (e.g. Week 4  $\psi_{LD}$ : LD 14:10 =  $0.28 \pm 0.06$  h, LD 19:5 =  $2.75 \pm 0.08$  h; Kuskall-Wallis Test;  $p < 0.001$ ).

On the day of the dark probe, more than 85% of animals displayed activity onsets that were advanced by less than 30 min relative to that observed during the preceding week. When the difference between activity onset during Week 3 and on the day of the dark probe was calculated, LD 14:10 and LD 19:5 were not significantly different ( $t(53) = 1.04$ ,  $p > 0.3$ ). Both these observations serve to verify that photoperiod-dependent differences in  $\alpha$  and  $\psi_{LD}$  were not a product of negative masking by light.

Table 2

Wheel running revolutions (in thousands) in Experiment 2

	<i>h</i> (0–5 <sup>a</sup> )	<i>h</i> (5–12)	<i>h</i> (12–24)	<i>h</i> (0–24)
LD 14:10-DIM	4.5 ± 1.1	8.1 ± 1.3	3.3 ± 0.9	16.0 ± 2.9
LD 14:10-DARK	4.7 ± 1.2	9.2 ± 1.4	2.0 ± 0.9	16.0 ± 2.8
LD 14:10-DIM+L	4.5 ± 0.7	3.6 ± 0.8 <sup>b</sup>	9.1 ± 1.0 <sup>b</sup>	15.8 ± 1.4
LD 14:10-DARK+L	4.4 ± 0.7	5.9 ± 0.8	5.6 ± 1.0	15.8 ± 1.5
LD 19:5-DIM	6.8 ± 0.9	7.3 ± 0.9	4.0 ± 0.9 <sup>b</sup>	21.2 ± 2.2
LD 19:5-DARK	6.2 ± 0.9	6.8 ± 0.9	8.2 ± 0.9	18.1 ± 2.2

<sup>a</sup> Hour (*h*) 0 = time of transfer to wheel running cages.

<sup>b</sup> Levels different from DARK cohort ( $p < 0.1$ ; see text).

#### 3.2.2. Wheel running during the first 24 h after transfer

Following transfer, animals within all groups engaged in robust wheel running during the first 5 h after transfer (Fig. 4, Table 2). Wheel running levels tended to taper off and then rise once more several hours later. LD 14:10 and LD 19:5-DARK animals, but not LD 19:5-DIM animals, discontinued wheel running shortly after the initial novelty-induced activity bout (Fig. 4). Relative to their DARK counterparts, 19:5-DIM animals displayed a long bout of novelty-induced activity after transfer ( $p < 0.05$ ; Fig. 4B) and did not run robustly at a phase consistent with their prior entrainment ( $p < 0.05$ ; Fig. 4B; Table 2). After resumption of wheel running, LD 14:10 animals receiving the bright light pulse exhibited wheel running patterns similar to those of LD 14:10-DARK and -DIM groups, with the exception that the former animals exhibited less activity during the 7 h light pulse and a large increase in wheel running during late subjective night (compare Fig. 4A and C, Table 2). Relative to their DARK cohorts also receiving a light pulse, LD 14:10-DIM+L animals tended to show less wheel running during the pulse ( $p = 0.08$ ; Table 2) and a larger increase in subsequent wheel running ( $p < 0.05$ ; Table 2).

#### 3.2.3. Phase shifts and $\tau$ under constant conditions

The magnitude of phase shifts depended on dim illumination and photoperiodic history, in addition to the type of manipulation provided (Fig. 5). Nonphotic phase shifts exhibited by LD 14:10-DARK and LD 14:10-DIM animals were

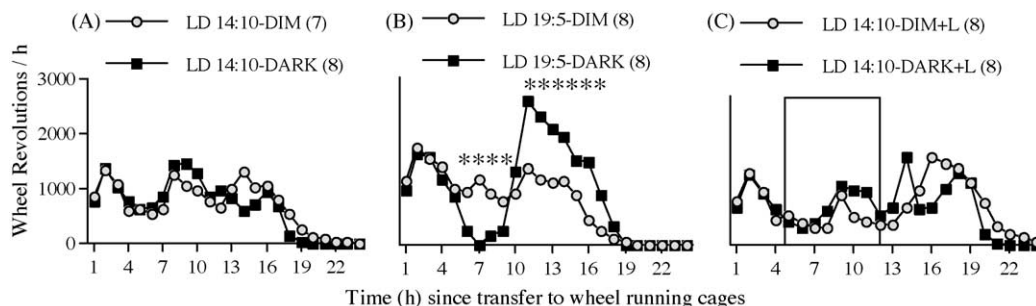


Fig. 4. Mean hourly revolutions across the 24 h after transfer to wheel running cages in Experiment 2. (A) and (B) LD 14:10 and LD 19:5 groups were transferred to cages with constant dim illumination (-DIM) or darkness (-DARK). Asterisks signify an hour at which LD 19:5-DARK activity was significantly different from that of LD 19:5-DIM ( $p < 0.05$ ). (C) LD 14:10 groups were transferred to cages with constant dim light (-DIM) or darkness (-DARK) and received a 7 h bright light pulse (+L) beginning 5 h after transfer (represented by the internal box). The number in parentheses is the number of animals per group.



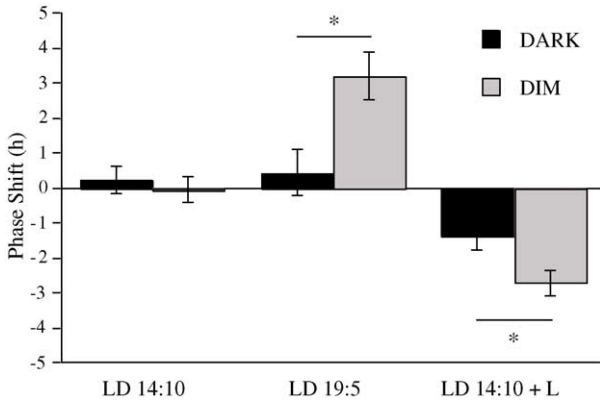


Fig. 5. Phase shifts ( $\pm$ S.E.M.) elicited during Experiment 2, as determined by the difference in post-transfer wheel running onsets and the PIR onset displayed during entrainment. Phase advances and delays are plotted as positive and negative values, respectively. Asterisks signify significant differences between DIM and DARK groups ( $p < 0.05$ ).

negligible, and no difference due to DIM light was evident ( $t(13) = 0.57, p > 0.5$ ). In contrast, phase advances exhibited by LD 19:5-DIM animals were  $\sim 3$  h larger than those exhibited by their DARK counterparts ( $t(14) = -2.97, p < 0.05$ ). Additionally, DIM light significantly enhanced the magnitude of phase delays exhibited by LD 14:10 animals receiving NWR followed by a 7 h bright light pulse ( $t(14) = 2.12, p = 0.05$ ). When phase shifts were instead calculated relative to activity onset on the day of the dark probe or to ZT12 (rather than to the Week 4 average activity onset), these results were upheld (data not shown). No significant differences in  $\tau$  were evident between groups in the two weeks after release into constant conditions. Group means ranged from 23.89–24.07 h.

### 3.3. Experiment 3

#### 3.3.1. Emergence of phase jumps

Dim illumination accelerated the expression of a phase jump, a response that was exhibited ultimately by all animals (Fig. 6). Four animals with DIM light displayed phase jumps within three weeks of nighttime scotophase reductions, and the remaining DIM animals initiated phase jumps over the next several weeks. In contrast, DARK animals exhibited phase jumps only after the nighttime scotophase was reduced to 3.5 h. As a result, DIM animals phase jumped while the nighttime scotophase was longer relative to DARK animals (survival analysis,  $\chi^2(1) = 14.77; p < 0.001$ ) and over a significantly broader range of nighttime scotophases (DIM: 6.5–3.0 h; DARK: 3.5–2.5 h; Kuskall-Wallis test,  $\chi^2(1) = 3.72; p < 0.001$ ).

#### 3.3.2. Entrainment before and after the emergence of phase jumps

In addition to the marked effect on the emergence of phase jumps, scotopic illumination affected entrainment early in the study, when animals were transferred from LD 14:10 to LD 17:7. On the week under LD 17:7, activity bouts of DIM animals were shorter relative to their DARK counterparts, ( $\alpha$ : DIM =  $8.19 \pm 0.27$ ; DARK =  $9.2 \pm 0.28$ ;  $p < 0.05$ , LS means contrast) and phased closer to the nighttime lights-off transition ( $\psi_{LD}$ : DIM =  $1.05 \pm 0.24$ ; DARK =  $2.22 \pm 0.24$ ;  $p < 0.05$ , LS means contrast). In the following week under the matching skeleton photoperiod, however, group differences disappeared ( $p > 0.05$ , LS mean contrasts), and over the next two weeks, DIM and DARK animals continued to entrain to skeleton photoperiods similarly ( $p > 0.05$ , LS mean contrasts). As the majority of DIM animals displayed phase jumps over the subsequent weeks, differences in en-

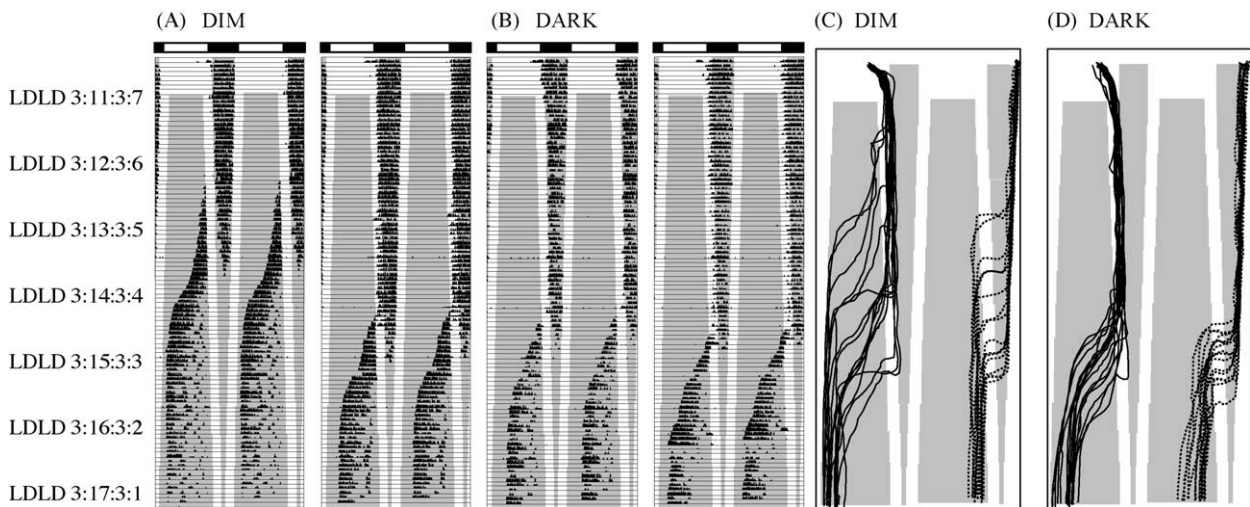


Fig. 6. Entrainment to the series of skeleton photoperiods simulating increases in day length in Experiment 3, where scotophases were marked by dim illumination (DIM) or complete darkness (DARK). (A) and (B) Representative double-plotted wheel-running actograms for two separate animals from DIM and DARK groups, respectively. (C) and (D) Tracings of activity rhythms exhibited by individual animals within DIM and DARK groups. Each line represents a separate animal. For clarity, onsets and offsets are shown disjointed on separate representations of the double-plotted photocycle. Labels at the far left represent the skeleton photoperiod provided every other week of the experiment, and the last digit indicates the length of the nighttime scotophase.



trainment were not assessed beyond the fourth week of the experiment.

After the initiation of a phase jump, the phase of wheel running continued to realign into the daytime scotophase, and a phase jump was noted to be complete when no activity remained within the nighttime scotophase. Once a phase jump was initiated, the latency to realignment was significantly longer under DIM conditions (DIM:  $12.67 \pm 1.37$  days; DARK:  $4.88 \pm 1.37$  days;  $t(30) = -4.03$ ,  $p < 0.001$ ).  $\alpha$  for the week preceding the phase jump, however, did not differ between animals in DIM and DARK conditions ( $5.95 \pm 0.41$  and  $5.93 \pm 0.41$ , respectively;  $t(30) = -0.03$ ,  $p > 0.9$ ). After phase jump completion,  $\alpha$  expanded within the daytime scotophase and at the end of the experiment, DIM animals displayed longer  $\alpha$  than their DARK cohorts (DIM:  $10.99 \pm 0.34$ ; DARK:  $7.93 \pm 0.35$ ;  $t(30) = -6.33$ ,  $p < 0.001$ ).

#### 4. Discussion

Far from being biologically inefficacious, dim illumination of an intensity comparable to dim moonlight and starlight can markedly alter circadian phase resetting and entrainment, as demonstrated here across three different experiments. As previously reported for male Syrian hamsters [15], dim light facilitated LDLD-induced splitting in females. All animals housed with dimly lit scotophases exhibited split rhythms in Experiment 1, but this was not a secondary consequence of increases in the amount of nonphotic feedback. DARK animals housed with standard wheels were less active than their DIM counterparts but not significantly so, which could reflect differences in the age or sex of hamsters used in the present study [15]. More importantly, provision of modified wheels increased wheel running levels above those of DIM animals but failed to elicit split rhythms in all but one dark-exposed animal. Although dim illumination may augment wheel running, its facilitation of LDLD-induced splitting would not seem to be a mere product of increased activity levels.

The dim illumination used presently (around 0.004 lux) is well below previously reported photic requirements for phase resetting and melatonin suppression in the hamster. Of these responses, the latter is the most sensitive, with previously reported light thresholds ranging from 1.1 to 0.08 lux [6,7,30,31]. Consistent with these published fluence-response curves, discrete 1 h dim light pulses during early or late subjective night (CT 14 and CT 18) did not induce phase shifts among animals free running in otherwise constant darkness (unpublished observations). Furthermore, melatonin-dependant photoperiodic responses were intact under short day photoperiods incorporating comparable scotopic illumination [13].

If scotopic illumination facilitates LDLD-induced splitting independent of activity levels and classic circadian responses to photic stimuli, in what manner could it operate?

Experiment 2 examined whether dim light renders animals more responsive to nonphotic and photic resetting. Transfer of wheel-naïve animals to wheel running cages induced large phase advances in LD 19:5-DIM animals only. Since no facilitation of nonphotic phase shifting was observed after entrainment to LD 14:10, these effects of dim light may be limited to animals entrained to photoperiods with short nights. One caveat to the interpretation that dim illumination enhances nonphotic sensitivity after LD 19:5 is that relative to their dark cohorts, LD 19:5-DIM animals ran for a longer time after transfer to wheel running cages. However, this may be a consequence, rather than a cause of their larger phase advances. Existing intensity–response curves, collected under admittedly different conditions, saturate at wheel-running levels accomplished by animals in Experiment 2 within the first 5 h after transfer [4,37]. Further, the phase of the circadian pacemaker is generally reset within a few hours of exposure to photic and nonphotic zeitgebers [23]. Thus, the extended activity in LD 19:5-DIM animals may represent a continuity between NWR-induced and phase-shifted circadian activity. In support of this point, activity offset on this first day after transfer also appears to be advanced in LD 19:5-DIM animals relative to their DARK cohorts.

Dim light also increased the magnitude of phase delays elicited by NWR followed by a long bright light pulse. Because differential phase resetting was not observed for LD 14:10-entrained animals after NWR alone, augmented phase delays after the compound stimulus likely resulted from dim light interacting with the bright light stimulation during early subjective night. Experiment 3, which focused on photic cues and minimized novelty-induced activity, also indicated that dim light modulates light-induced resetting. Specifically, after the abrupt change from LD 14:10 to LD 17:7, DIM animals displayed a less positive  $\psi_{L/D}$  and shorter  $\alpha$  relative to DARK animals.

Because photoperiodic compression of  $\alpha$  has been implicated in LDLD-induced splitting [12,15], Experiment 3 primarily investigated whether dim light would influence re-entrainment to skeleton photoperiods simulating increases in day length. Scotopic illumination unambiguously accelerated the emergence of a phase jump under these conditions. Phase jump responses observed presently were consistent with previous reports for this species in that activity of all animals advanced into the daytime scotophase [34,41]. However, in the absence of a formal understanding of precisely how phase jumps emerge, it is difficult to specify the mechanisms through which dim illumination accelerates this response. Previous models largely account for phase jumps through an asymmetry in delay and advance regions of the photic PRC [34,38,41]. These early models, however, do not take into account photoperiod-induced changes in the amplitude of the photic PRC, now known to be correlated with  $\alpha$  [32,36]. During entrainment to ultra long day lengths, like those simulated in Experiment 3, light-induced phase shifts are markedly attenuated and thus less clearly able

to generate phase jumps in this manner. In multi-oscillator models of the circadian pacemaker, the coupling between component oscillators also changes as a function of  $\alpha$  [35], and these changes may underlie the expression of phase jumps. Phase jumping under skeleton photoperiods may provide an additional paradigm under which dim light could exert its effect by altering  $\alpha$  and the interactions between oscillators. A common mechanism could underlie both phenomena, as a “minimum tolerable night” near 5 h characterizes both LDLD-induced splitting (unpublished observations) and phase jumping [Experiment 3, [34,38,41]]. If this were indeed the case, then the fact that phase jumping was ultimately observed in all DARK animals would predict that split rhythms would emerge under LDLD cycles with completely dark nights if shorter scotophases were provided.

Considering the results from the present three studies, it is now possible to address the role of dim light in promoting split rhythms under LDLD. The case is perhaps clearest for the animals of Experiment 1 that were unsplit prior to Interval 2. During Interval 1, these animals had activity largely confined to the 5 h nighttime scotophase and were thus effectively entrained to a skeleton LD 19:5, near the threshold for phase jumps for DIM animals in Experiment 3. The cage change at the beginning of Interval 2 provides a nonphotic zeitgeber similar to that given to LD 19:5 animals in Experiment 2, which produces little effect unless dim illumination is provided. Thus, animals with scotopic illumination are more responsive to both photic and nonphotic factors operating under LDLD during Interval 2. In contrast, there is little impetus for dark-exposed animals to alter entrainment, since DARK animals are expected neither to be phase advanced by NWR during the cage change (Experiment 2) nor rendered susceptible to phase jumps under a skeleton of LD 19:5 (Experiment 3). These results are consistent with a proposed model of LDLD-induced splitting in which novelty-induced activity induces large phase advances of distinct populations of circadian oscillators [15].

Nonphotic phase resetting, however, does not seem to underlie the dim-enhancement of LDLD splitting during Interval 1. As shown in Experiment 2, dim illumination and darkness do not differentially influence nonphotic phase resetting in animals previously entrained to LD 14:10. Instead, the critical interaction occurring after the initial transfer to wheel running cages may be that between the dim and bright light exposure. By augmenting photic phase delays, scotopic illumination may enhance  $\alpha$  compression under LDLD, similar to its effect after transfer to LD 17:7 in Experiment 3. Photoperiod-induced compression of subjective night may then increase the likelihood of a phase jump and thereby promote splitting in dim-exposed animals. Dim light interacting with photic phase resetting during Interval 1 and nonphotic phase advances during Interval 2 could provide the impetus for the two waves of splitting observed under LDLD (c.f. Fig. 2). There were notable differences between intervals in the pattern of split rhythm emergence (i.e. gradual

versus abrupt), similar to previous reports using male hamsters [15]. Additionally, splitting initiated during Interval 2 appeared to be less stable than that which emerged during Interval 1. Only further study can determine whether these patterns derive from the different photoperiodic histories of animals splitting in Intervals 1 and 2 or unknown intrinsic differences in the circadian function of these behaviorally distinguished hamster groups.

Dim illumination could influence re-entrainment under the present paradigms by changing the waveform or amplitude of the photic PRC. The modicum of evidence collected thus far indicates that dim light does not interact with bright light in a uniform manner. For example, when the 3 h light pulse scanned subjective night during phase jumping, this did not cause a more rapid realignment of activity rhythms in DIM animals. On the contrary, the transition to the daytime scotophase took significantly longer under dimly lit nights. An interaction between dim and bright light, moreover, is perhaps unable to explain the full suite of effects demonstrated thus far. Following transfer from long to short day lengths, dim light accelerated re-entrainment [13], which is achieved primarily through means other than bright light-induced phase shifts [16]. Dim light can certainly affect both nonphotic and photic resetting but given that these interactions appear to be limited to specific conditions (e.g. certain photoperiods), we suggest that dim light's fundamental action lies elsewhere. Collectively, these data are consistent with the hypothesis that dim illumination alters circadian waveform by modulating the interactions, or coupling, between component oscillators. Further study of dim light may clarify its underlying physiology and potentially that of circadian coupling.

Dim illumination, below established thresholds for phase shifting and melatonin suppression, can nonetheless modulate biological rhythmicity. While these data challenge current assumptions about the photic sensitivity of the circadian pacemaker, this is not without precedent. In addition to its acute effect on activity levels [8,10,15,27], dim light has been found to influence circadian entrainment in other nocturnal mammals [9,24]. Kavanau reported that mice will entrain to dim:dark cycles (<0.02 lux) with activity during the dim light phase, although this lacked rigorous quantification [22]. Additionally, dim illumination has been used under the context of studies with twilight transitions, which widen the range of photic entrainment in hamsters and mice [5,21]. Use of scotopic illumination also permits entrainment to T cycles well beyond the “normal” circadian range [17]. While these studies of dim light employ lighting conditions never experienced in nature, marked effects of dim light are found also under simpler paradigms (i.e. transfer from long to short photoperiods [13] and Experiment 2). Convergent effects of dim light across laboratory paradigms in multiple species attest to its potency as a modulator of circadian rhythms. It remains to be determined, however, whether illumination from the moon and stars—with its specific spectral and temporal characteristics—markedly alters circadian processes in nature.

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## References

- [1] Aschoff J. Response curves in circadian periodicity. In: Aschoff J, editor. *Circadian clocks*. Amsterdam: North Holland; 1965, p. 95–111.
- [2] Aschoff J, Figala J, Poppel E. Circadian rhythms of locomotor activity in the golden hamster (*Mesocricetus auratus*) measured with two different techniques. *J Comp Physiol Psychol* 1973;85:20–8.
- [3] Biberman LM, Dunkelman L, Fickett ML, Finke RG. Levels of nocturnal illumination. Washington, DC: Institute for Defense Analyses, Research, and Engineering Support Division; 1966.
- [4] Bobrzynska KJ, Mrosovsky N. Phase shifting by novelty-induced running: activity dose–response curves at different circadian times. *J Comp Physiol [A]* 1998;182:251–8.
- [5] Boulos Z, Macchi MM, Terman M. Twilights widen the range of photic entrainment in hamsters. *J Biol Rhythms* 2002;17:353–63.
- [6] Brainard GC, Richardson BA, Hurlbut EC, Steinlechner S, Matthews SA, Reiter RJ. The influence of various irradiances of artificial light, twilight, and moonlight on the suppression of pineal melatonin content in the Syrian hamster. *J Pineal Res* 1984;1:105–19.
- [7] Brainard GC, Richardson BA, Patterborg LJ, Reiter RJ. The effect of different light intensities on pineal melatonin content. *Brain Res* 1982;233:75–81.
- [8] Edelstein K, Mrosovsky N. Behavioral responses to light in mice with dorsal lateral geniculate lesions. *Brain Res* 2001;918:107–12.
- [9] Erkert HG, Bay FA, Kracht S. Zeitgeber induced modulation of activity patterns in nocturnal mammals (*Chiroptera*). *Experientia* 1976;32:560–2.
- [10] Erkert HG, Grober J. Direct modulation of activity and body temperature of owl monkeys (*Aotus lemurinus griseimembra*) by low light intensities. *Folia Primatol (Basel)* 1986;47:171–88.
- [11] Evans JA, Gorman MR. Split circadian rhythms of female Syrian hamsters and their offspring. *Physiol Behav* 2002;76:469–78.
- [12] Gorman MR. Exotic photoperiods induce and entrain split circadian activity rhythms in hamsters. *J Comp Physiol [A]* 2001;187:793–800.
- [13] Gorman MR, Elliott JA. Dim nocturnal illumination alters coupling of circadian pacemakers in Siberian hamsters *Phodopus sungorus*. *J Comp Physiol [A]* 2004;190:631–9.
- [14] Gorman MR, Elliott JA. Entrainment of 2 subjective nights by daily light:dark:light:dark cycles in 3 rodent species. *J Biol Rhythms* 2003;18:502–12.
- [15] Gorman MR, Elliott JA, Evans JA. Plasticity of hamster circadian entrainment patterns depends on light intensity. *Chronobiol Int* 2003;20:233–48.
- [16] Gorman MR, Freeman DA, Zucker I. Photoperiodism in hamsters: abrupt versus gradual changes in day length differentially entrain morning and evening circadian oscillators. *J Biol Rhythms* 1997;12:122–35.
- [17] Gorman MR, Kendall ME, Elliott JA. Scotopic illumination enhances entrainment of circadian rhythms to lengthening light:dark cycles. *J Biol Rhythms* 2005;20:38–48.
- [18] Gorman MR, Lee TM. Daily novel wheel running reorganizes and splits hamster circadian activity rhythms. *J Biol Rhythms* 2001;16:541–51.
- [19] Gorman MR, Yellon SM, Lee TM. Temporal reorganization of the suprachiasmatic nuclei in hamsters with split circadian rhythms. *J Biol Rhythms* 2001;16:552–63.
- [20] Johnson CH, Elliott JE, Foster R. Entrainment of circadian programs. *Chronobiol Int* 2003;20:741–73.
- [21] Kavanau JL. Activity and orientational responses of white-footed mice to light. *Nature* 1968;218:245–52.
- [22] Kavanau JL. Behavior of captive white-footed mice. *Science* 1967;155:1623–39.
- [23] Mead S, Ebling FJ, Maywood ES, Humby T, Herbert J, Hastings MH. A nonphotic stimulus causes instantaneous phase advances of the light-entrainable circadian oscillator of the Syrian hamster but does not induce the expression of c-fos in the suprachiasmatic nuclei. *J Neurosci* 1992;12:2516–22.
- [24] Meijer JH, Daan S, Overkamp GJ, Hermann PM. The two-oscillator circadian system of tree shrews (*Tupaia belangeri*) and its response to light and dark pulses. *J Biol Rhythms* 1990;5:1–16.
- [25] Michel S, Colwell CS. Cellular communication and coupling within the suprachiasmatic nucleus. *Chronobiol Int* 2001;18:579–600.
- [26] Mrosovsky N. Locomotor activity and the non-photic influences on circadian clocks. *Biol Rev Cambridge Soc* 1996;71:343–72.
- [27] Mrosovsky N, Foster RG, Salmon PA. Thresholds for masking responses to light in three strains of retinally degenerate mice. *J Comp Physiol [A]* 1999;184:423–8.
- [28] Mrosovsky N, Janik D. Behavioral decoupling of circadian rhythms. *J Biol Rhythms* 1993;8:57–65.
- [29] Mrosovsky N, Salmon PA, Vrang N. Revolutionary science: an improved running wheel for hamsters. *Chronobiol Int* 1998;15:147–58.
- [30] Nelson DE, Takahashi JS. Comparison of visual sensitivity for suppression of pineal melatonin and circadian phase-shifting in the golden hamster. *Brain Res* 1991;554:272–7.
- [31] Nelson DE, Takahashi JS. Integration and saturation within the circadian photic entrainment pathway of hamsters. *Am J Physiol* 1999;277:R1351–61.
- [32] Pittendrigh C, Elliott J, Takamura T. The circadian component in photoperiodic induction. *CIBA Foundation Symp* 1984;104:26–47.
- [33] Pittendrigh CS. Circadian organization in cells and the circadian organization of multicellular systems. In: Schmitt, FO, Worden, FG, editors. *The neurosciences: third study program*. Cambridge: MIT Press, 1974, p. 437–458.
- [34] Pittendrigh CS, Daan S. A functional analysis of circadian pacemakers in nocturnal rodents: IV Entrainment: pacemaker as clock. *J Comp Physiol* 1976;106:291–331.
- [35] Pittendrigh CS, Daan S. A functional analysis of circadian pacemakers in nocturnal rodents: V Pacemaker structure: a clock for all seasons. *J Comp Physiol* 1976;106:333–55.
- [36] Pohl H. Strain differences in responses of the circadian system to light in the Syrian hamster. *Experientia* 1983;39:372–4.
- [37] Reeb SG, Mrosovsky N. Large phase-shifts of circadian rhythms caused by induced running in a re-entrainment paradigm: the role of pulse duration and light. *J Comp Physiol [A]* 1989;165:819–25.
- [38] Sharma VK, Singaravel M, Chandrashekar MK, Subbaraj R. Relationship between free-running period and minimum tolerable light pulse interval of skeleton photoperiods in field mice *Mus booduga*. *Chronobiol Int* 1997;14:237–45.
- [39] Shirakawa T, Honma S, Honma K. Multiple oscillators in the suprachiasmatic nucleus. *Chronobiol Int* 2001;18:371–87.
- [40] Sinclair SV, Mistlberger RE. Scheduled activity reorganizes circadian phase of Syrian hamsters under full and skeleton photoperiods. *Behav Brain Res* 1997;87:127–37.
- [41] Stephan FK. Circadian rhythms in the rat: constant darkness, entrainment to T cycles and to skeleton photoperiods. *Physiol Behav* 1983;30:451–62.
- [42] Thorington L. Actinic effects of light and biological implications. *Photochem Photobiol* 1980;32:117–29.