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CHRONOBIOLOGY INTERNATIONAL
Vol. 20, No. 2, pp. 233–248, 2003

Plasticity of Hamster Circadian Entrainment Patterns Depends on Light Intensity

Michael R. Gorman,[1,*] Jeffrey A. Elliott,[2] and Jennifer A. Evans[1]

[1]Department of Psychology and [2]Department of Psychiatry, University of California, San Diego La Jolla, California, USA

ABSTRACT

The multiple oscillatory basis of the mammalian circadian pacemaker is adduced by, among other phenomena, the occurrence of split locomotor activity rhythms in rodents after prolonged exposure to constant light. More recently, split rhythms entrained to a 24h light:dark:light:dark cycle have been documented following scheduled access of hamsters to a novel running wheel or by photoperiod manipulations alone. Because the incidence of constant light-induced splitting depends on light intensity, the role of this variable was assessed in this new splitting paradigm. Male Syrian hamsters, entrained to a 14h light:10h dark cycle, were transferred to individual running wheel cages 7h after light onset. Transfer coincided with the beginning of the scotophase of a new photocycle alternating between 5h of relative dark and 7h of light. For four weeks bright photophases (~350 lux) were alternated with either dim (<0.1 lux) or completely dark (0 lux) scotophases. An additional group received moderate intensity photophases (~45 lux) paired with dim scotophase illumination. For an additional four weeks, all hamsters were exposed to the same bright:dim light:dark cycle. Dim light in the scotophase significantly increased the incidence of split activity rhythms relative to that observed with completely dark scotophases. Overall wheel-running rates and activity induced by a cage change were also increased in dim light-exposed animals. Group differences largely disappeared four weeks later when hamsters previously maintained in completely dark scotophases were exposed to dim scotophases. Photophase light intensity did not affect the overall incidence of splitting, but

*Correspondence: Michael R. Gorman, 9500 Gilman Drive, San Diego La Jolla, CA 92093-0109, USA; Fax: 858-534-7190; E-mail: mgorman@ucsd.edu.

DOI: 10.1081/CBI-120018576 0742-0528 (Print); 1525-6073 (Online)

influenced the timing of activity in the afternoon scotophase. The effects of dim illumination may be mediated in part via enhanced locomotor responses to transfer to a new cage or by changes in coupling interactions between component oscillators.

Key Words: Splitting; Coupling; Entrainment; Oscillator interactions; Light intensity effects; Circadian rhythm.

INTRODUCTION

In mammals, circadian rhythms are governed by a master pacemaker located in the suprachiasmatic nuclei (SCN) of the anterior hypothalamus. In the absence of environmental cues, circadian rhythms drift with respect to the sidereal day, but they may be entrained to match the 24h environmental light:dark cycle by a corrective phase-resetting action of light. A hallmark feature of the circadian system is its differential entrainment by seasonally varying light:dark cycles to produce behavioral and physiological rhythms appropriate to each phase of the year (Duncan, 1998; Elliott and Tamarkin, 1994; Pittendrigh and Daan, 1976b; Schwartz et al., 2001; Sumova et al., 1995). Moreover, in constant conditions the circadian pacemaker retains the influence of the prior entraining photoperiod as reflected in the rhythm's free-running period, waveform, and response to resetting stimuli (Pittendrigh and Daan, 1976a; Pittendrigh et al., 1984). These effects of photoperiodic history on pacemaker function may be readily explained if the pacemaker comprises two or more interacting oscillators (i.e., a complex pacemaker) (Daan and Berde, 1978; Oda et al., 2000; Pittendrigh and Daan, 1976b). A more direct argument for the existence of two interacting circadian pacemakers derives from the observation that hamster locomotor activity rhythms split into two components after prolonged exposure to constant light (LL) (Earnest and Turek, 1982; Pittendrigh, 1967; Turek et al., 1982). Although these components initially free run with different periods, they adopt a common free-running period when they reach an antiphase relationship.

Recently, we demonstrated a remarkable plasticity in circadian entrainment patterns adopted by hamsters exposed to 24h light:dark:light:dark (LDLD) cycles. Using a variety of paradigms, a substantial fraction of hamsters could be made to entrain their circadian rhythms to express markers of subjective night in both of two daily dark periods (i.e., scotophases) that were separated by a minimum of 5h of light. In contrast to these "split" circadian rhythms, other hamsters entrained to the LDLD cycles with markers of subjective night were restricted to only one of the daily scotophases (Evans and Gorman, 2002; Gorman, 2001; Gorman and Lee, 2001). By one method, daily transfer to darkened novel running wheels from ZT4-9 resulted in progressive delays in the nighttime home-cage activity onset (Gorman and Lee, 2001; Mrosovsky and Janik, 1993). After delays in activity onset of approximately 5h, hamsters that remained at home in a LDLD cycle thereafter exhibited split activity rhythms with one activity component expressed in the latter half of the night and a second component phased to coincide with the afternoon dark phase. Corresponding to these two intervals of increased locomotor activity, hamsters also exhibited two daily periods of elevated melatonin secretion and responsiveness to light pulses

as measured by behavioral phase-shifting and by induction of c-Fos immunoreactivity in the SCN (Gorman et al., 2001). Comparable entrainable split rhythms, moreover, can be generated without transfer to novel wheel cages; if the nighttime scotophase is shortened to 5h and a second 5h scotophase is added to the prior subjective day, a majority of hamsters will readily entrain with activity divided between the two daily scotophases (Gorman, 2001). Combining these two approaches, we discovered that rhythms could be split nearly instantaneously by transferring hamsters to wheel-running cages in the circadian afternoon and simultaneously instituting a LDLD7:5:7:5 photoperiod (Elliott and Gorman, unpublished observations).

In nocturnal rodents, classical LL-induced splitting is facilitated by increasing light intensities (Cheung and McCormack, 1983; Pickard et al., 1993; Puchalski and Lynch, 1991), whereas the opposite dependence on light intensity has been noted in a diurnal species (Hoffmann, 1971). Because light has been proposed to influence coupling between component circadian oscillators (Daan and Berde, 1978; Meijer et al., 1990) and because this novel form of splitting appears to reflect a change in oscillator interactions, we asked whether the incidence of split rhythms obtained under our LDLD paradigm would be altered by light schedules of different intensity. Specifically, we examined the influence of very dim light (< 0.1 lux) vs. total darkness during the scotophase. Additionally, we assessed the influence of photophase light intensity at two values (45 vs. 350 lux) generally sufficient to entrain and phase-shift hamster circadian rhythms.

METHOD

Animals

Male Syrian hamsters (HsdHan:AURA, Harlan, Indianapolis, IN), 5–6 weeks of age, were group-housed in polypropylene cages ($48 \times 27 \times 20$ cm) without running wheels in 14h of light and 10h of dark daily (LD14:10, lights off 21:00h PST—Pacific Standard Time) with food (Purina Rodent Chow #5001, St. Louis, MO) and water ad libitum. Daytime illumination was provided by 40W fluorescent bulbs that generated 100–300 lux at the cage lid. No illumination was provided at night.

Procedure

At 4–5 months of age, hamsters were transferred to individual cages ($27 \times 20 \times 15$ cm) equipped with running wheels (diameter = 17 cm). Running cages were contained in individual light-tight ventilated chambers. Transfer occurred between 13:45 and 14:10h PST with a new scotophase initiated at 14:00h. Thereafter hamsters remained on a LD7:5 photoperiod (lights out 02:00–07:00h; 14:00–19:00h PST), equivalent to a 24h LDLD7:5:7:5 cycle. Hamsters were randomly assigned to one of three groups that differed in light intensity of the photophases or scotophases, respectively. In Group Bright/Dim ($n = 13$), new 4W fluorescent bulbs generated photophase light intensities of 352 ± 5.7 lux (mean ± sem, $n = 13$), and green LEDS (0.03W) provided

<0.1 lux in the scotophase, both measured at the level of the cage lid. In Group Moderate/Dim ($n = 13$), light intensity of the photophase was reduced with shade cloth to 44.6 ± 0.8 lux ($n = 13$), but animals received the same dim green illumination in the scotophase. In Group Bright/Dark ($n = 13$), the dim LEDs were extinguished to provide absolute darkness during the scotophase and unshaded bulbs (350 ± 5.3 lux, $n = 13$) illuminated the photophases. Photophase and scotophase light intensities were determined with a Sper Scientific Light Meter Model 840020 (Scottsdale, AZ).

Hamsters were left in their cages relatively undisturbed except for cage changes every 13–14d, always performed in darkness with the aid of a red flashlight during the first 90min of the afternoon scotophase. Water and food were replenished at the same time. Four weeks into the study, animals previously housed under Moderate/Dim and Bright/Dark conditions were exposed to Bright/Dim conditions beginning with the scheduled cage change. All hamsters, including those initially maintained in Bright/Dim, remained under these conditions for an additional four weeks. For analytic purposes, the eight-week experiment was thus divided into four two-week intervals, each spanning the time from the initial transfer or cage change until the following cage change.

Rhythm Monitoring and Analyses

Wheel-running behavior triggered mechanical switches, and electrical closures were compiled into 6min bins by Dataquest III hardware (Mini-mitter, Bend, OR). Wheels triggered a single count every half revolution. Unfiltered actograms were prepared using ClockLab software (Actimetrics, Evanston, IL).

Locomotor activity patterns were analyzed separately during each experimental interval. Rhythms were classified as split if hamsters expressed activity (>1000 counts) in both daily scotophases for at least 5 of the 13–14d. To further characterize group differences in the temporal distribution of activity, hourly activity counts were averaged between hamsters for the 96h following cage changes. For statistical analyses of running patterns in the first days of each interval, activity counts of each animal were summed over each 5h scotophase and each 7h photophase. Lastly, overall rates of locomotor activity were assessed by calculating the total daily activity counts averaged over the 13–14d interval.

The role of scotophase illumination was assessed by contrasting activity patterns of Bright/Dim vs. Bright/Dark hamsters. The effect of photophase light intensity was assessed by comparing Bright/Dim and Moderate/Dim conditions. Because Moderate/Dim and Bright/Dark groups differed along two dimensions, no direct comparisons between these groups were made. Two-group comparisons were generally performed with the Students T-test (Statview 5.0, SAS Institute, Cary, NC). Where significant heterogeneity of variance was detected between groups (for example, when most or all subjects in one group failed to generate any activity counts at a particular time point), nonparametric Mann-Whitney U Tests were run, and these values are reported instead. The incidence of splitting was compared between groups with Chi-square analysis.

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RESULTS

Patterns of Entrainment and Incidence of Splitting

Hamsters transferred to LDLD7:5:7:5 exhibited a variety of unsplit and split activity patterns as depicted in Fig. 1. Hamsters classified as split expressed activity in each of the two daily scotophases (Fig. 1A, B), in some cases doing so from the first scotophase when they were transferred to wheel-running cages. In other cases classified as unsplit, activity was consistently restricted to the scotophase corresponding to the animal's previous night (Fig. 1C). In still other cases, hamsters first exhibited unsplit rhythms, but splitting occurred in association with a cage change (Fig. 1D, E) or a cage change and simultaneous change in lighting conditions such as occurred at the beginning of Interval 3 (Fig. 1F).

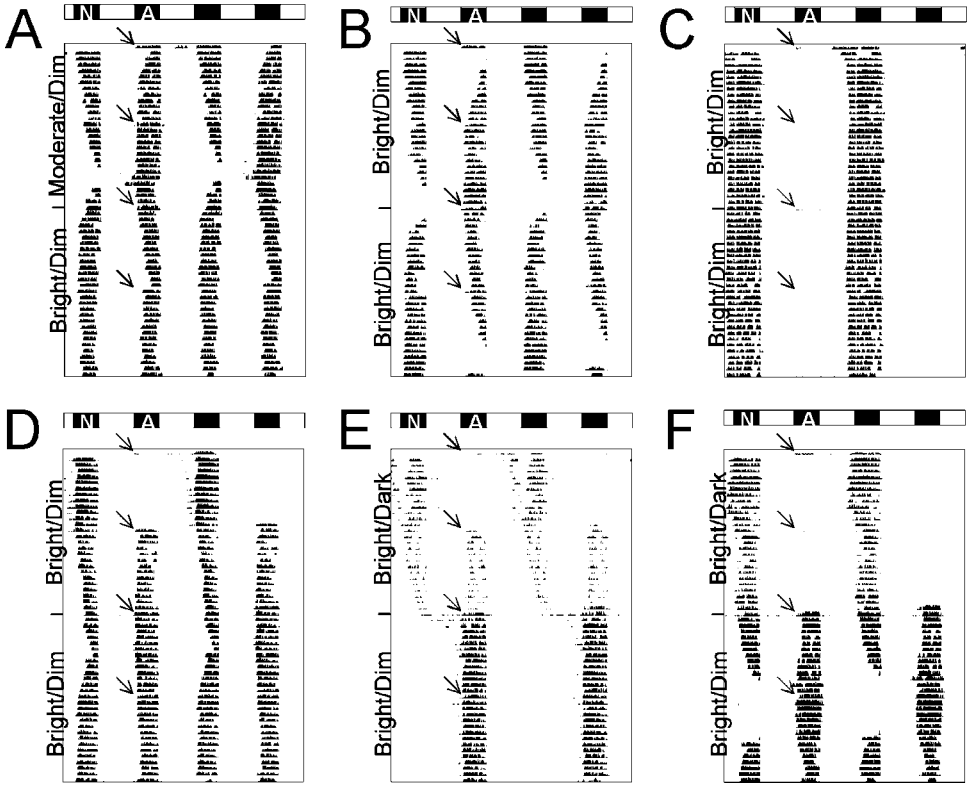

Figure 1. Representative double-plotted actograms (8 weeks long) from each of the three experiment groups. LDLD cycles are denoted above the figure with dark bars representing the scotophases. The afternoon (A) and nighttime (N) scotophases are indicated in their respective shaded areas. Y axis is scaled from 0 to 150 counts/min. Arrows indicate time of transfer to wheel cages and all subsequent cage changes that began Intervals 2, 3, and 4. Light intensity conditions are indicated along the left margin of actograms.

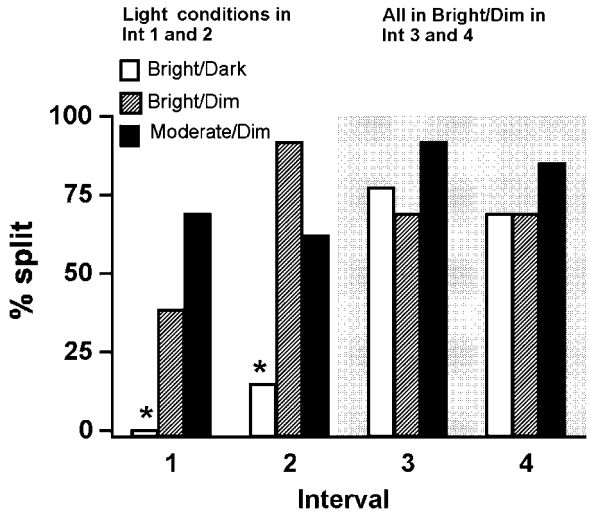

Figure 2. Incidence of splitting by treatment groups (n = 13/group) in each of the four experimental intervals. Note that all groups were maintained under identical Bright/Dim conditions in Intervals 3 and 4. Asterisk denotes that group differs in splitting incidence compared to Bright/Dim hamsters in that interval ($p < 0.05$).

Finally, some hamsters exhibited split activity profiles which later rejoined into unsplit rhythms (Fig. 1E).

The incidence of splitting depended on lighting conditions (Fig. 2). In both Intervals 1 and 2, hamsters in Bright/Dark were less likely to be split than were those exposed to Bright/Dim ($\chi^2(1) = 6.2$; $p < 0.05$ for Interval 1; $\chi^2(1) = 15.5$; $p < 0.001$ for Interval 2). Moderate vs. bright light, however, did not influence splitting incidence among dim light-exposed hamsters ($\chi^2(1) = 2.5$; $p > 0.10$ for Interval 1; $\chi^2(1) = 3.5$; $p > 0.05$ for Interval 2). When all hamsters were exposed to Bright/Dim in Intervals 3 and 4, group differences in splitting incidence disappeared as those unsplit hamsters previously housed in Bright/Dark were largely induced to split.

Activity Levels

Lighting conditions also significantly influenced overall running rates (Fig. 3A). In Intervals 1 and 2, hamsters completed approximately 50% more wheel revolutions each day if exposed to dim light in the scotophase ($p < 0.001$, Bright/Dim versus Bright/Dark). Bright vs. moderate light intensity during the photophase, however, did not affect overall running rates ($p > 0.50$). Higher activity counts among dim light-exposed hamsters were not a result of the higher incidence of splitting in dim light as might be expected if animals were running in two daily scotophases instead of just one. In Interval 1, for instance, nonsplitting hamsters still ran significantly more in Bright/Dim than did nonsplitting hamsters in Bright/Dark (mean daily counts: 26935 ± 1450, $n = 8$ vs. 18906 ± 1573, $n = 13$, respectively, $p < 0.001$). When all animals were exposed to Bright/Dim in

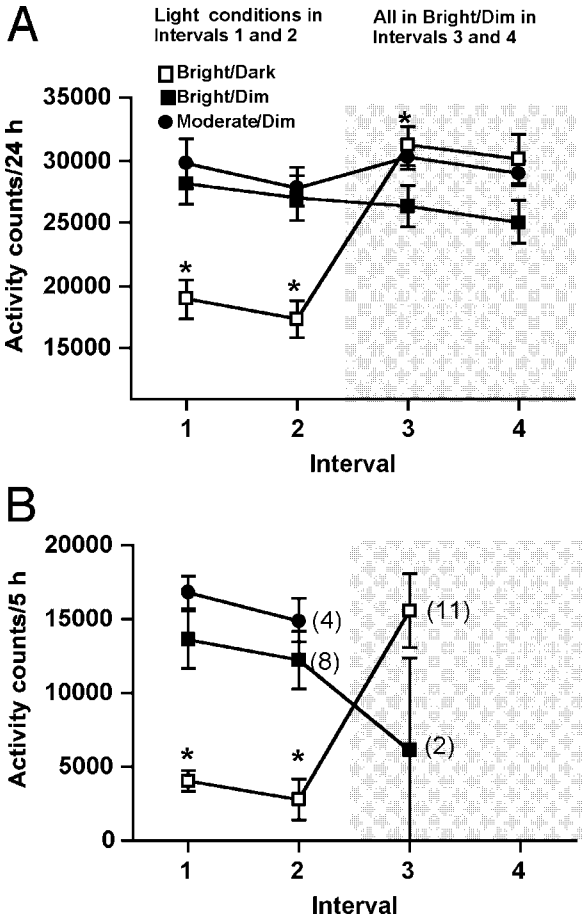

Figure 3. Mean (± sem) daily activity counts of hamsters from three experimental groups (A; n = 13/group) and mean (± sem) activity counts induced in the 5h afternoon scotophase associated with a cage change (B). To highlight activity specifically induced by the cage change, the bottom figure includes data from only those hamsters not expressing afternoon activity in the days prior to the cage change. Sample size per group is 13 except as noted in parentheses. Too few hamsters were unsplit before Interval 4 to be included. Conventions as in Fig. 2.

Intervals 3 and 4, group differences were largely eliminated, although those initially exposed to Bright/Dim ran significantly less than those previously maintained in Bright/Dark ($p < 0.05$).

The wheel-running activity provoked in the 5h afternoon scotophase of each cage change was also significantly affected by lighting conditions. Dim scotophase illumination tripled the number of wheel revolutions in the first 5h after transfer to wheel cages in Interval 1 (Fig. 3B; $p < 0.001$ Bright/Dim versus Bright/Dark). Likewise, the cage change that began Interval 2 provoked markedly more activity among dim- vs. dark-exposed hamsters among the subset of hamsters that had been inactive in the final afternoon

scotophases of Interval 1 (Fig. 3B; $p < 0.001$ Bright/Dim vs. Bright/Dark). Among the nonsplitters in Bright/Dark, moreover, the cage change into dim illumination in Interval 3 acutely induced comparably high levels of afternoon wheel running four weeks into the experiment (Fig. 3B).

Dynamics of Re-entrainment

Figure 4 shows activity profiles averaged between all animals of a group for the first four days of Interval 1. Hamsters in Bright/Dim ran at markedly higher rates in the first afternoon scotophase compared to those in Bright/Dark ($p < 0.01$; U-Test). Those in Bright/Dark, however, ran robustly in the following evening photophase (19:00–02:00h PST), whereas Bright/Dim hamsters ran significantly less ($p < 0.01$). Bright/Dim hamsters, however, ran significantly more than Bright/Dark hamsters in the subsequent nighttime scotophase (02:00–07:00h PST; $p < 0.01$). Neither group ran during

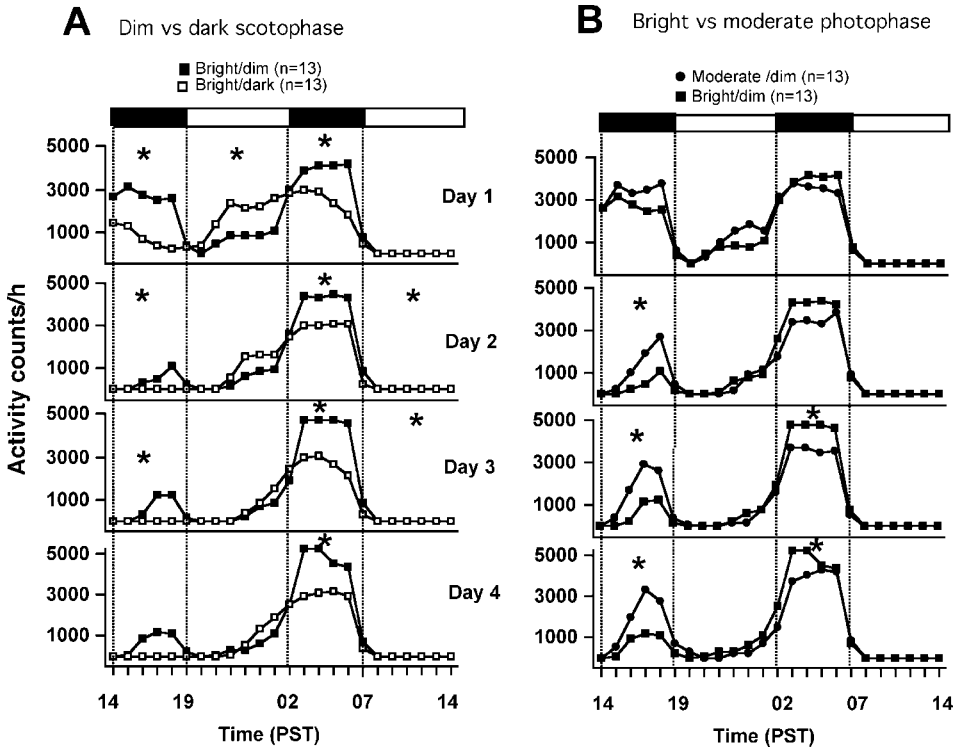

Figure 4. Mean hourly activity counts for the first 4d of Interval 1. The role of scotophase light intensity is assessed by comparing Bright/Dark versus Bright/Dim hamsters (A), and the role of photophase light intensity is illustrated by contrasting Bright/Dim versus Moderate/Dim groups (B). For figure clarity, standard errors are not shown. Asterisks indicate statistically significant ($p < 0.05$) group differences in activity integrated over the scotophase or photophase. Conventions as in Fig. 1.

the morning photophase (07:00–14:00h PST) beyond the modest activity seen in the first hour. In the afternoon scotophase of Day 2, Bright/Dim hamsters ran more than did Bright/Dark hamsters ($p < 0.01$; U-test). This pattern of group differences was largely preserved on subsequent days of the experiment (Fig. 4A).

Hamsters exposed to moderate versus bright photophase light intensities showed similar patterns of activity in the first 24h (Fig. 4B) with no differences observed until the afternoon scotophase of Day 2. In this afternoon scotophase, and in subsequent afternoons, Moderate/Dim hamsters ran significantly more than did Bright/Dim hamsters ($p < 0.05$). Moderate/Dim hamsters, however, ran less than Bright/Dim hamsters in the nighttime scotophases of Days 3 and 4 ($p < 0.05$).

Because compression of the subjective night had previously been proposed to facilitate splitting (Gorman, 2001), two analyses were undertaken post-hoc to assess whether splitting emerged differently in hamsters previously entrained to express subjective night in a 10h versus a 5h scotophase. First, we contrasted the emergence of

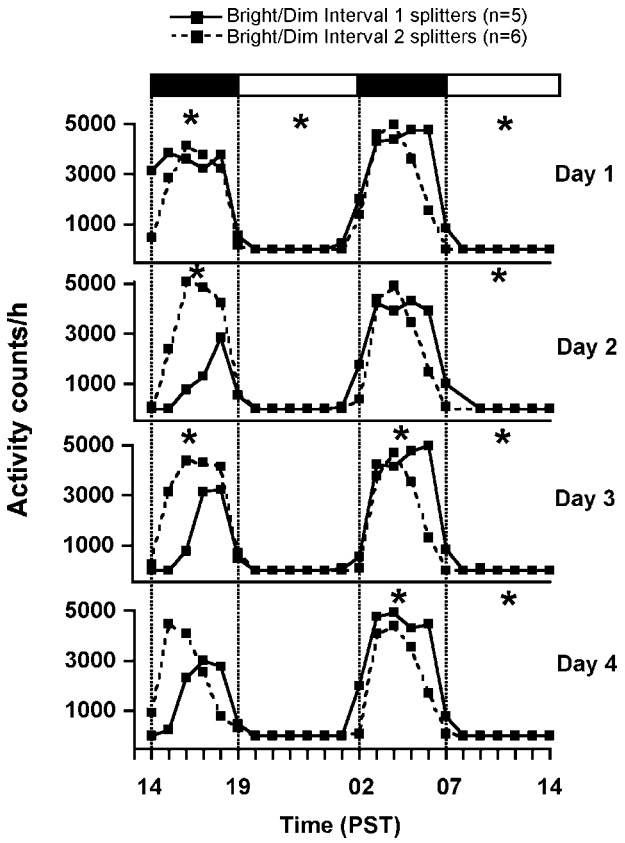

Figure 5. Mean hourly activity counts of Bright/Dim hamsters induced to split after transfer to running cages at the onset of Interval 1 or after the cage change that began Interval 2. Conventions as in Fig. 4.

split rhythms of Bright/Dim hamsters that split in Interval 1 (n = 5) with those that first appeared in Interval 2 (n = 6). Animals splitting in Interval 1 ran slightly more in three of the four phases of the first day of Interval 1 than did splitting animals in the first 24h of Interval 2 (Fig. 5). On subsequent days, however, Interval 2 splitters ran substantially more in the afternoon and substantially less in the nighttime scotophases compared to Interval 1 splitters in the corresponding times of Interval 1.

With the same guiding rationale, we compared the response of previously unsplit hamsters transferred from pre-experimental LD14:10 to Bright/Dim in Interval 1 with that obtained from unsplit Bright/Dark hamsters transferred into Bright/Dim in Interval 3. Unsplit Bright/Dark hamsters exposed to Bright/Dim for the first time in Interval 3 exhibited significantly more afternoon activity on Days 2, 3, and 4 ($p < 0.001$; Fig. 6) than

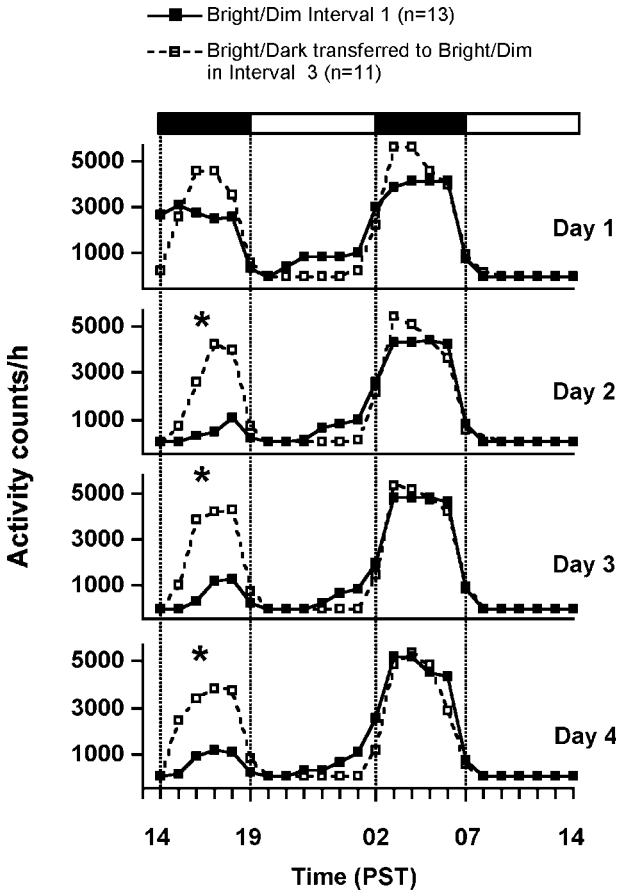

Figure 6. Mean hourly activity counts of hamsters transferred from pre-experimental conditions to Bright/Dim in Interval 1 or from Bright/Dark to Bright/Dim at the beginning of Interval 3. Two Bright/Dark hamsters that had already split in Interval 2 were excluded from this analysis. Conventions as in Fig. 5.

did the Bright/Dim hamsters in Interval 1. No other differences reached statistical significance.

DISCUSSION

Transfer of wheel-naïve hamsters from LD14:10 to LDLD7:5:7:5 resulted in the rapid establishment of split locomotor activity rhythms characterized by the presence of wheel-running in each of two daily scotophases. In general, these rhythms resembled the split rhythms described in more detail in previous studies in which splitting was induced by daily exposure to novel wheel running (paired with darkness) (Gorman and Lee, 2001), by home-cage photoperiod manipulations alone (Gorman, 2001), or by maternal communication of split circadian rhythms (Evans and Gorman, 2002). It should be noted that these split rhythms differ in important respects from those induced by prolonged exposure to constant light. Specifically, whereas the LL-induced rhythms reflect antiphase oscillations of the left and right SCN (de la Iglesia et al., 2000), these LDLD-entrained split rhythms exhibit no sign of asymmetric physiological organization (Gorman et al., 2001). Like LL-induced split rhythms, however, the expression of these LDLD-entrained split rhythms depends on light intensity: dim light during the scotophase markedly enhances the incidence of splitting, while intensity of photophase illumination influenced the appearance of afternoon activity without affecting overall splitting incidence.

An unanticipated outcome of this study was the elevated levels of running among dim light-exposed hamsters and the particularly enhanced acute response of dim light-exposed hamsters to transfer into wheel-running cages and to cage changes. Enhanced locomotor activity in response to dim illumination has not, to our knowledge, been reported in hamsters. In mice, however, dim light (<1 lux) early in the night acutely increases wheel-running activity in animals maintained in light:dark cycles that otherwise provide total darkness for the scotophase (Edelstein and Mrosovsky, 2001). This effect, which in mice depends on the integrity of the classical image-forming visual system, was reportedly absent in hamsters under comparable conditions (Redlin and Mrosovsky, 1999; Redlin et al., 1999). It is unclear what accounts for the apparent discrepancy with the present findings. Exposures to dim light longer than 1h may be necessary for facilitatory effects of dim light. Alternatively, in studies that reported negative findings, running wheels were wrapped in plastic mesh. As mesh wrapping markedly increases running rates overall (Mrosovsky et al., 1998), lower baseline running levels from unwrapped wheels may be necessary to observe effects of dim light on motivation to run or on physical coordination.

Are the effects of dim light on splitting incidence and on activity levels related at a causal level? Although it is clear that the increased running of dim light-exposed hamsters is not a simple consequence of the fact that split hamsters are running in two, rather than one, scotophases daily, a potential effect of induced activity on splitting is more difficult to define precisely. Certainly, increased afternoon activity induced by a cage change would be expected to facilitate splitting. In several previous studies that employed daily exposure to novel running wheels, the amount of novelty-induced running predicted whether hamsters would exhibit split rhythms in the home cage (Evans and Gorman, 2002; Gorman and Lee, 2001; Mrosovsky and Janik, 1993). Accordingly, in the present study, splitting often occurred in association with cage changes. Because the present experimental design

did not attempt to eliminate running induced by cage changes, it is not possible to assess rigorously whether dim light could facilitate splitting by other mechanisms—for example, by altering coupling relations of component circadian oscillators. Circumstantial evidence, however, suggests that it does: whereas dark-exposed hamsters never split apart from a cage change, several dim light-exposed hamsters split 1 or more days following a cage change (e.g., Fig. 1B). In other studies, moreover, splitting emerged reliably in the absence of afternoon activity provoked by a cage change. Photoperiod changes alone were sufficient to split rhythms even when these manipulations began days apart from any cage change (Gorman, 2001). Further work on dim nighttime illumination is warranted as this has received scant attention (c.f. Daan and Pittendrigh, 1976) despite evidence that it substantially alters entrainment and free-running behavior in some species (Erkert et al., 1976; Meijer et al., 1990).

In previous work with novel wheel access, the proportion of activity shifted from the nighttime to the afternoon scotophase was directly related to the number of days of scheduled novel wheel running (Gorman and Lee, 2001). To account for this and other findings, we proposed that each day of scheduled novel wheel exposure induced very large phase-shifts of subsequent cohorts of component oscillators such that the subjective night of individual components was shifted to coincide with the afternoon rather than the nighttime scotophase. Comparably large shifts of the entire circadian system have been noted in animals given novel wheel cages in DD (Gannon and Rea, 1995), and in animals given sufficient days of scheduled novel wheel access (Gorman and Lee, 2001). In other words, novel wheel running appears to "pull" some component oscillators apart from what was otherwise a stable, unsplit system. The present results support this type of model: one group of animals transferred from pre-experimental LD14:10 to wheel cages at the beginning of the experiment (Bright/Dark) ran robustly in the subsequent photophase at a time that would be predicted based on their prior entrainment to LD14:10 (i.e., projected early subjective night) but not during the afternoon scotophase. In contrast, animals that exhibited a novel wheel effect in the first scotophase of the experiment (i.e., Bright/Dim animals) showed remarkably less activity at this time of projected early subjective night. While simple fatigue after running in the afternoon might also account for this result, the fact that Bright/Dim hamsters ran significantly more in the subsequent scotophase argues against this explanation. Instead, running in the afternoon scotophase likely shifted some oscillators that would otherwise have mediated activity early in subjective night. In both previously reported novel wheel- and photoperiod-induced splitting paradigms, the split afternoon activity bout likewise emerged at the expense of evening activity (Gorman, 2001; Gorman and Lee, 2001). This model of splitting by rapid phase-shifting of a subpopulation of oscillators is additionally supported by the appearance of activity in afternoon scotophase of Day 2.

By contrast, the extreme compression of the nighttime scotophase appears to contribute to splitting by creating a highly unstable entrainment state (Gorman, 2001; Pittendrigh and Daan, 1976a). If a second scotophase is introduced in the subjective day of the unsplit system, the system may reorganize by entraining subjective nights in each of the two dark periods. In an earlier experiment, this reorganization from exclusively nighttime to split afternoon and nighttime activity occurred gradually until a new split equilibrium was reached (Gorman, 2001). In that study, therefore, compression of the subjective night rather than induced afternoon activity appeared to

be the proximate trigger of splitting, although effects of a cage change were noted in two animals exposed to the photoperiod paradigm. Finally, in both paradigms, light intervening between the two scotophases counteracts the tendency of the two oscillatory components to rejoin, as indicated by the fusion that is readily observed after transfer to DD.

It is in the context of these prior results that the effects of light history on splitting emergence are best understood. In Bright/Dim, the emergence of splitting was more abrupt if hamsters had been previously entrained to LDLD7:5:7:5 (i.e., those Bright/Dim hamsters failing to split in Interval 1 and those kept in Bright/Dark until Interval 3). In these cases, transfer to a new cage induced intense afternoon running that would be expected to phase-shift component oscillators. Additionally, as all of these hamsters had been entrained to a very short subjective night, their entrainment state was likely highly unstable and thus poised to split. Together these two factors would be expected to facilitate rapid splitting. In contrast, prior to Interval 1 the subjective nights of hamsters were not compressed and thus not unstable. Transfer of Bright/Dim animals to wheel-running cages induced activity, but like the more modest effects of one day of novel wheel running in other paradigms (Janik et al., 1994), this was unlikely on its own to be sufficient to induce an even split of the circadian system. Compression of the subjective night was achieved, however, in the days that followed, which likely contributed to a delayed splitting.

But why was afternoon activity greater early in Interval 1 among Moderate/Dim versus Bright/Dim hamsters? As noted above, the entire circadian pacemaker can be phase-advanced by many hours in a single day if animals are transferred to new cages in constant darkness (Gannon and Rea, 1995) or in association with a shift of the entire light:dark cycle (Mrosovsky and Salmon, 1987). When daily novel wheel running is presented in a light:dark cycle, this instantaneous large phase-shift of the entire pacemaker is thus counteracted by the photophase(s) that follows and/or precedes it, although incremental phase-shifting of component oscillators nonetheless persists with additional days of scheduled afternoon activity. Outside of the context of splitting, moreover, attenuation of novel wheel-induced phase shifts by subsequent light pulses has been clearly documented (Biello and Mrosovsky, 1995; Mrosovsky, 1991). Splitting may be facilitated in Moderate/Dim because light of approximately 45 lux is less effective than brighter light (350 lux) in attenuating the phase-shifts mediated by induced running. Consequently, a greater proportion of oscillators may be rapidly shifted by induced activity in 45 lux compared to 350 lux. As these light intensities are well above the threshold for entraining unsplit hamster rhythms and may indeed be comparable for phase-shifting the unsplit pacemaker (Nelson and Takahashi, 1991), their differential effect on the kinetics of splitting warrants further explicit testing.

In summary, the Syrian hamster circadian system exhibits impressive plasticity in entrainment mediated by interactions between component oscillators. The generality of this specific splitting phenomenon is not known. Nonetheless, our results suggest that an understanding of these interactions may be of practical use for interventions related to shiftwork, jetlag, and circadian disorders. Relatively modest changes in light intensity are sufficient to markedly alter these interactions, warranting future work on light intensity, specifically as it relates to the complex pacemaker.

ACKNOWLEDGMENTS

We are grateful to Antonio Mora and Tony Mora for excellent animal care and to two anonymous reviewers for their helpful comments on the manuscript. This research was supported by NIH grants HD-36460, NS-30235, and NSF grant IBN-998567.

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Received September 1, 2002
Returned for revision October 13, 2002
Accepted October 31, 2002