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ORIGINAL PAPER

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Exotic photoperiods induce and entrain split circadian activity rhythms in hamsters

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Abstract The split circadian activity rhythm that emerges in hamsters after prolonged exposure to constant light has been a theoretical cornerstone of a multi-oscillator view of the mammalian circadian pacemaker. The present study demonstrates a novel method for splitting hamster circadian rhythms and entraining them to exotic light:dark cycles. Male Syrian hamsters previously maintained on a 14-h day and 10-h night were exposed to a second 5-h dark phase in the afternoon. The 10-h night was progressively shortened until animals experienced two 5-h dark phases beginning 10 h apart. Most hamsters responded by splitting their activity rhythms into two components associated with the afternoon and nighttime dark phases, respectively. Each activity component was entrained to this light:dark:light:dark cycle. Transfer of split hamsters to constant darkness resulted in rapid joining of the two activity components with the afternoon component associated with onset of the fused rhythm. In constant light, the nighttime component corresponded to activity onset of the fused rhythm, but splitting emerged again at an interval characteristic for this species. The results place constraints on multi-oscillator models of circadian rhythms and offer opportunities to characterize the properties of constituent circadian oscillators and their interactions.

Keywords Oscillator · Photoperiod · Coupling · Splitting · Circadian

Abbreviations *DD* constant dark · *E* evening oscillator · *LDLD* light:dark:light:dark cycle · *LL* constant light · *M* morning oscillator · *NWR* novel wheel running · *SCN* suprachiasmatic nuclei · *ZT* zeitgeber time

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Introduction

The splitting of circadian rhythms into two distinct components has been a cornerstone upon which a dual- or multi-oscillator view of the mammalian circadian pacemaker is based. After prolonged exposure to constant light (LL) activity duration of Syrian hamsters (*Mesocricetus auratus*) typically shortens, and the activity rhythm can split into two distinct components (Boulos and Rusak 1982; Boulos and Terman 1979; Hoffmann 1971; Pickard et al. 1993; Pittendrigh 1967; Pittendrigh and Daan 1976b; Turek et al. 1982). Because the two components are sometimes traceable to evening and morning activity fractions of the unsplit rhythm, the oscillators presumed to underlie these distinct components are sometimes designated evening (E) and morning (M), respectively (Earnest and Turek 1982; Pittendrigh and Daan 1976b). With different freerunning periods in LL, τ_E and τ_M, the two components eventually reach an anti-phase relationship after which they are recoupled and freerun together with a fixed-phase relationship and an altered period. Why splitting occurs is not exactly known, but changes in coupling between oscillators may result from differential effects of light on the two oscillators. If coupling is disrupted by LL, the two oscillators may freerun independently until coupling is restored in the anti-phase relationship, which is more stable against the LL background. Split rhythms in SCN firing rates in vitro support the hypothesis that the two oscillators physically coexist within this structure (Margraf et al. 1991; Mason 1991). Anti-phase oscillations of clock-gene expression has recently been demonstrated in the left and right SCN of LL-induced split hamsters (de la Iglesia et al. 2000).

On largely independent empirical grounds, dual-oscillator models have also been invoked to explain the seasonal modulation of various circadian rhythms. Seasonal compression and expansion of the duration of hamster wheelrunning behavior, melatonin secretion and phase-resetting by light depend on the phase angle of E and M oscillators (Daan and Berde 1978; Elliott and Tamarkin 1994; Gorman et al. 1997; Illnerova and Vanacek 1985; Pittendrigh and Daan 1976b). The E oscillator, named to suggest its role in generating activity, melatonin secretion, etc. associated with the early hours of night, is entrained by delaying phase shifts of evening light. Correspondingly, M is thought to generate the same traits as E but to do so late in the night with entrainment effected by phase-advances of the morning light transition. If τ_E and τ_M in darkness are less and greater than 24 h, respectively, E and M will drift apart until they abut their respective light transitions. By this mechanism, the phase angle between them (Ψ_{E-M}) will be increased in long nights of winter and decreased in summer, thereby accounting for the seasonal modulation of various circadian traits. As a left/right suprachiasmatic nuclei (SCN) basis for E and M is not suspected in the unsplit state, no physiological basis for these oscillators has yet been established (Daan et al. 2001). Nevertheless, the seasonal adjustment of SCN neuropeptide expression and Fos-inducibility by light establishes that photoperiodic modulation occurs with the SCN itself (Duncan et al. 1995; Sumova et al. 1995).

Although both photoperiodism and splitting suggest dual component circadian oscillators, these two areas of research have remained largely separate no doubt because the conditions favorable for splitting, LL, are incompatible with photoperiodism studies. Recently, however, split rhythms of nocturnal mammals were reported in constant darkness (DD) and in a light:dark:light:dark (LDLD) cycle (Gorman and Lee 2001; Mrosovsky and Janik 1993). The more recent study was prompted by the finding of Mrosovsky and Janik (1993) that 17 days of novel wheel running (NWR) from ZT5–8 split the activity rhythms of male Syrian hamsters transferred to DD. A refined NWR procedure was shown to induce split rhythms, which entrained to a LDLD cycle with robust entrained activity in each of the two daily scotophases (Gorman and Lee 2001). For each split activity component, moreover, light pulses delivered 1 h after activity onset induced both phase-delays of the pulsed activity component and Fos-immunoreactivity in the SCN (Gorman et al. 2001). These results clearly established that the circadian pacemaker contains component oscillators, which may be temporally reorganized to express two short subjective nights roughly 180° out of phase.

In the course of an entrainment study of NWR-induced split rhythms, a hamster with an unsplit rhythm was simultaneously exposed to a progressive shortening of the night and addition of a 5-h afternoon scotophase. Surprisingly, this hamster underwent splitting although not exposed to NWR. This led me to test whether manipulations of photoperiod alone could generate reliable splitting in a larger group of hamsters. The study reported here reproduces the conditions serendipitously discovered to induce splitting in that initial hamster. The resultant split rhythms of hamsters are described as are the patterns of rhythm rejoining observed in constant conditions.

Methods and materials

Fifteen male Syrian hamsters, *Mesocricetus auratus* (HsdHan: AURA, Harlan, Indianapolis, Ind.), 5–6 weeks of age were housed singly in LD 14:10 (lights off 1900 hours PST) for 6 weeks prior to this study. Food (Purina Rodent Chow No. 5001) and water were available ad libitum throughout the study. Hamsters were placed in polypropylene cages (27 cm×20 cm×15 cm) equipped with running wheels (diameter: 17 cm) located in individual light-tight ventilated chambers with light intensity of 50–100 lx at the bottom of the cage during the photophase and dim green illumination (< 1 lx) during the scotophase. On the day of transfer to these cages an afternoon scotophase (1100–1600 hours PST) was added to the ongoing LD 14:10 photocycle to yield an LDLD 6:5:3:10 photoperiod (initial lights on 0500 hours PST). After 4 days on this LDLD cycle, the 10-h scotophase was progressively shortened to 5 h via delays of lights off. For 11 hamsters, the afternoon scotophase was simultaneously delayed as illustrated in Fig. 1. Non-technical terminology used to describe each segment of the LDLD cycle is also indicated. For 4 additional control hamsters, the afternoon scotophase was eliminated after the first delay in the nocturnal lights off so that hamsters were housed in LD 19:5 after 4 days. Hamsters remained in these photoperiods for 12 weeks.

Demasking

Twelve weeks after reaching the final LDLD cycle, light pulse deletions were carried out on eight experimental hamsters which split under those light conditions. In a counterbalanced design, either the 9-h morning light pulse or the 5-h evening pulse was omitted for a single day with a minimum of 6 days between treatments. Four control hamsters in LD19:5 received a single 5-h scotophase in the afternoon corresponding to that of split hamsters.

To assess whether the split rhythms would persist or undergo fusion in constant conditions, five hamsters with continuously split rhythms and one hamster with an intermittently split rhythm were exposed to LL initiated by leaving lights on after the morning photophase. Four split hamsters (three continuously and one intermittently) were transferred to DD initiated after the nighttime scotophase.

Data analysis

Wheel-running activity was monitored by Dataquest III software (Mini-mitter, Sun River, Ore.), and activity counts were compiled into 10-min bins. Data analyses were carried out with Statview 5.0

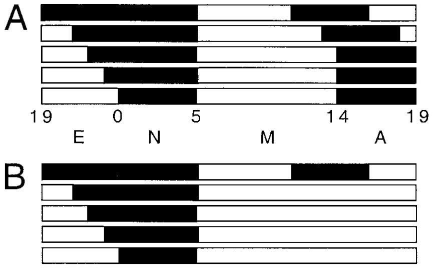

Fig. 1A,B Two photoperiodic manipulations used in this study. Each horizontal bar represents successive 24 h intervals each beginning at 1900 hours PST. *Filled rectangles* indicate times of darkness. The first photoregime denoted was in effect for 3 days prior and the photoregime denoted on the 5th day remained in effect for 12 weeks except as noted in the text. *E* and *M* designate evening and morning photophases, respectively. *A* and *N* represent afternoon and nighttime scotophases, respectively

(SAS Institute, Cary, N.C.) and ClockLab software (Actimetrics, Evanston, Ill.). Activity onset was defined as the point at which activity levels first rose above below 2.5 rev min^{-1}. Activity offset was the point at which activity level fell below 2.5 rev min^{-1} and remained so for 120 min. Activity duration (α) was calculated as the difference between activity offset and the previous onset. Phase angle of entrainment (Ψ_{onset}) was calculated as the difference between lights off and the activity onset associated with that scotophase. To understand the relation between the two activity components as splitting occurred, a series of Pearson product-moment correlation coefficients was calculated for each hamster. For the first 16 days of split rhythms, correlations of afternoon activity onset with both onset and offset of the nighttime activity bout were determined. Also examined were correlations between total afternoon and nighttime activity and between α of each component.

To assess the effects of deletion of the morning photophase, the following were evaluated (Fig. 6): (1) change in offset of the preceding nighttime activity ($\Delta N_{\mathrm{offset}}$) bout relative to the value of the same bout the day prior; (2) change in onset of the afternoon activity ($\Delta A_{\mathrm{onset}}$) bout relative to the day preceding the deleted scotophase; (3) change in the inactive period between nighttime and afternoon activity components ($\Delta\rho_{\mathrm{N-A}}$) relative to the day prior to the deletion; and (4) change in the subsequent nighttime activity onset ($\Delta A+1_{\mathrm{onset}}$) relative to the same value prior to the deletion. Analogous values ($\Delta A_{\mathrm{offset}}$, $\Delta N_{\mathrm{onset}}$, $\Delta\rho_{\mathrm{A-N}}$, $\Delta N+1_{\mathrm{onset}}$) were calculated to assess alterations in activity after deletions of the evening photophase. All were evaluated with one-sample *t*-tests (two-tailed).

Results

In LDLD 9:5:5:5, most hamsters (8 of 11) entrained with clearly split rhythms which did not change appreciably over the previous 10 weeks of exposure to this biphasic photocycle (Fig. 2). The remaining three hamsters also split their activity, although they did not show stable entrainment, and rhythms rejoined one or more times (Fig. 3). In contrast, hamsters exposed to LD 19:5 showed no evidence of split rhythms (Fig. 4). All entrained to LD 19:5 with a positive phase angle, which decreased with time in the photoperiod.

Entrainment parameters

After 12 weeks in their respective photocycles, split hamsters and unsplit controls showed markedly different patterns of entrainment to their respective scotophases (1). Ψ_{onset} was negative for both activity components of split hamsters. The nighttime activity component was significantly more negative than the afternoon component ($P < 0.001$). The afternoon activity component was

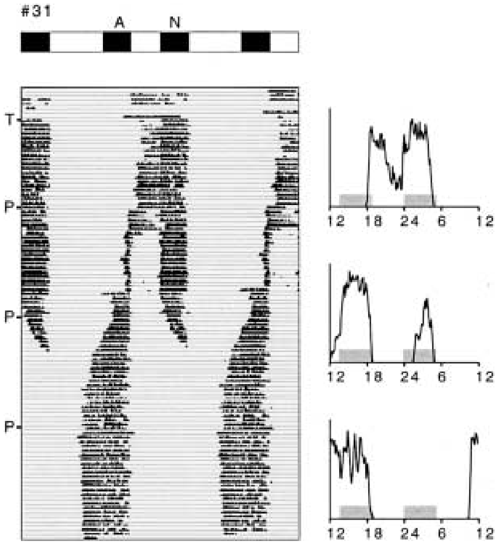

Fig. 3 Representative actogram and activity profiles of a hamster failing to entrain to the light:dark:light:dark (LDLD) cycle. Conventions as in Fig. 2

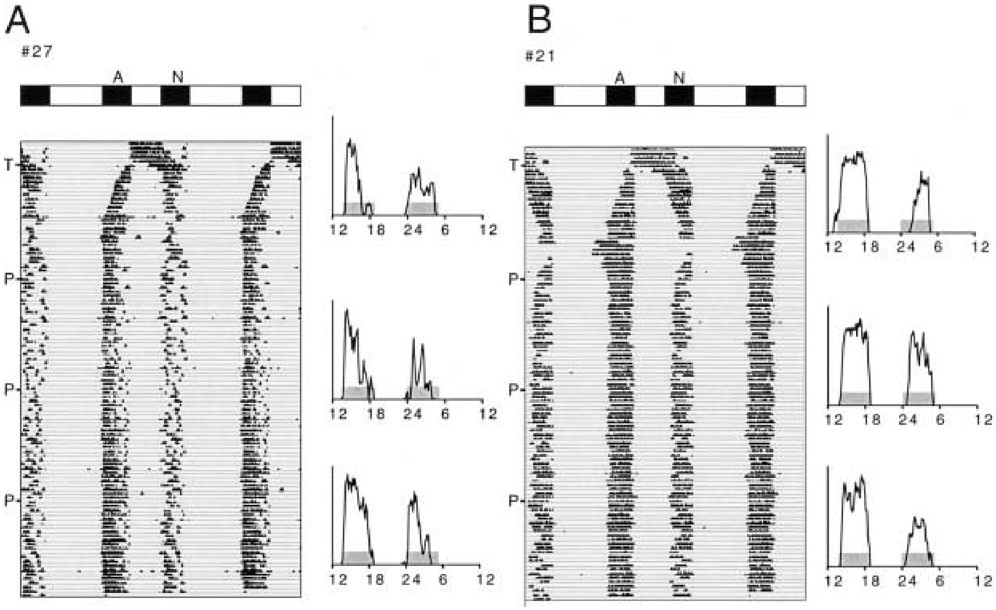

Fig. 2A,B Representative double-plotted actograms and 7-day average activity profiles of hamsters splitting in response to photoperiod manipulation. Data are unfiltered and scaled on the ordinate from 0 to the maximum number of wheel revolutions for that hamster. On the *left margin of actograms* are indicated the onset of photoperiod transitions (*T*) and the beginning of each 7-day interval averaged in the activity profiles (*P*). The light/dark cycle in effect after photoperiod transitions is depicted above actograms as described in Fig. 1. Activity profiles are singly plotted with dark periods represented by *shaded areas*. Conventions as in Fig. 1

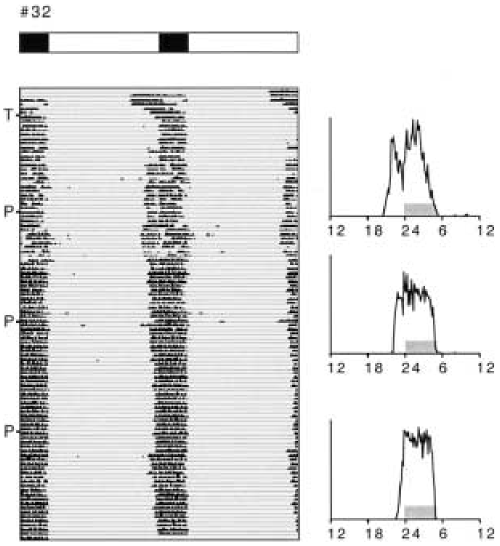

Fig. 4 Representative actogram and activity profile of a control hamster entraining to LD 19:5. Conventions as in Fig. 2

also characterized by a greater α and contained a greater fraction of the total activity (Table 1). In contrast, Ψ_{onset} and α of control hamsters were significantly greater than corresponding values of both split activity components. The total amount of activity per 24 h did not differ between split and control hamsters.

Initiation of splitting

Two patterns of splitting were noted among the eight hamsters with stable split rhythms. In six hamsters, an afternoon activity component emerged gradually beginning within 1–3 days of the shortening of the nighttime scotophase to 5 h (Fig. 5a). The incipient afternoon activity onset occurred always during the latter fraction of the afternoon scotophase and advanced earlier with successive days. For each of the hamsters exhibiting this pattern of rapid splitting, the onset of afternoon activity was significantly negatively correlated with the amount and duration of activity in that bout (i.e., earlier bouts were longer and contained more activity; data not shown). Correlated with advances of the emerging afternoon bout, *nighttime* activity onsets also progressively delayed. For each of these six animals, there were significant negative correlations between (1) onset of the afternoon and nighttime activity components; (2) the duration of activity in each component and (3) the total amount of activity in each component (Fig. 5a; Table 2). Finally, the onset of afternoon activity was not consistently correlated with offset of the nighttime component. Rather, these variables were uncorrelated in three hamsters, positively correlated in one, and negatively correlated in two. In the remaining two stable splitters, activity rhythms did not split until after 10 days in the LDLD 9:5:5:5. In both, splitting occurred immediately following a cage change during the afternoon scotophase (Fig. 5b). Prior to splitting, endogenous control of nocturnal activity onset may have been masked by the lengthening photophase; that is, masking may be suggested by the expression of much earlier activity onsets on the four days preceding the cage change that coincided with the emergence of splitting of hamster no. 29 (Fig. 5b). For both of the delayed splitting hamsters, splitting was associated with correlated changes in the parameters describing the afternoon and nighttime activity components.

Assessment of negative masking

Deletion of the morning photophase did not significantly affect the activity offset of the preceding nighttime activity bout (Fig. 6; $\Delta N_{\mathrm{offset}}$, $P > 0.20$) or the activity onset of the following afternoon ($\Delta A_{\mathrm{onset}}$, $P > 0.14$) despite trends towards delays and advances of these measures, respectively. Consequently, the inactive interval, $\Delta\rho_{\mathrm{N-A}}$, was reduced on the day the pulse was deleted (Fig. 6; $P < 0.06$). This latter difference became robustly significant ($P < 0.01$) when two additional hamsters were included that received identical treatment as they were transferred to DD 2 weeks later. The onset of the following nighttime activity component ($\Delta N + 1_{\mathrm{onset}}$) was unaffected by the deletion ($P > 0.15$).

Deletion of the evening photophase did not significantly extend the offset of the preceding afternoon activity bout (Fig. 6; $\Delta A_{\mathrm{offset}}$, $P > 0.35$) but did advance the onset of the following nighttime activity component ($\Delta N_{\mathrm{onset}}$, $P < 0.05$). Correspondingly, the inactive interval between activity components, $\Delta\rho_{\mathrm{A-N}}$, was shorter than on the day preceding the photophase deletion (Fig. 6; $P < 0.05$). The subsequent afternoon activity onset ($\Delta A + 1_{\mathrm{onset}}$) was unaffected ($P > 0.75$).

Table 1 Circadian rhythm parameters (mean ± SEM) of split activity rhythms (both components) and of unsplit control hamsters during the final week of entrainment

	Split hamsters				Unsplit controls	*n*
	Night-time	Afternoon		*n*		
ψ_{onset} (h)	−1.05 ± 0.11	−0.33 ± 0.13	$P < 0.001$	9	0.92 ± 0.20*	4
α (h)	2.93 ± 0.26	3.76 ± 0.25	$P < 0.05$	9	5.34 ± 0.30*	4
% Activity	32.5 ± 1.9	67.5 ± 1.9	$P < 0.05$	9		
Total daily activity			18755 ± 2101	9	16985 ± 4104	4

*Differs significantly from nighttime and afternoon values ($P < 0.01$)

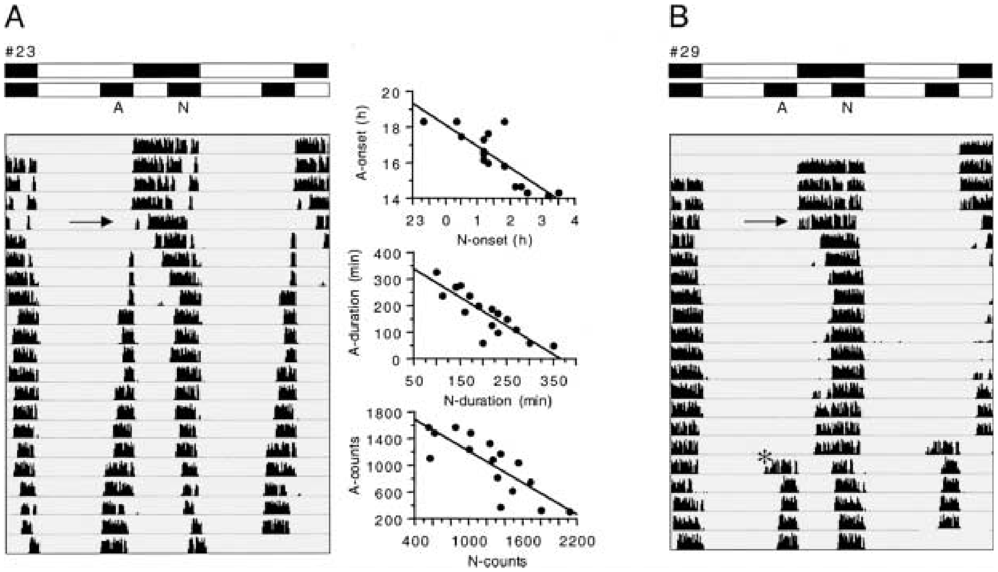

Fig. 5A,B Activity component analysis and actograms illustrating two patterns of splitting in response to photoperiod transitions. Above the actograms, the two LD regimes represent photoperiods during the first 4 days, and last 15 days of the actograms, respectively. **A** When the nighttime scotophase is first shortened (*arrow*), hamster no. 23 shows delays or masking of nighttime wheelrunning activity. Over the next several days, activity delays further but a second activity gradually emerges in the latter fraction of the afternoon scotophase. On the *right* are depicted correlational analyses of afternoon and nighttime activity onsets, durations and total activity over the first 16 days of split locomotor activity. **B** Activity rhythms are not split in hamster no. 29 immediately after photoperiod transitions, but split after a cage change (*asterisk*)

Addition of a single afternoon scotophase to hamsters entrained to LD 19:5 did not induce locomotor activity or alter subsequent rhythms in LD 19:5 (data not shown).

Split rhythms under constant conditions

Transfer to LL grossly suppressed activity levels, although clear circadian locomotor activity persisted. In every case, the split rhythm disappeared in LL and a unimodal rhythm appeared with an activity onset continuous with that of the afternoon component under entrained conditions (Fig. 7a, b). In three hamsters, a split rhythm reappeared after prolonged exposure to LL (30–50 days) and the two components free-ran coupled in an anti-phase relation (Fig. 7a).

In every case in DD, onset of the nighttime activity component phase-delayed on the 1st day of DD and either phase-advanced or phase-delayed on subsequent days (Fig. 7c, d). Afternoon activity onset uniformly phase-advanced until rhythms were not distinctly split. Activity offset of the afternoon component also clearly phase-advanced for the first 5 days in DD. In all cases, activity onset of the fused rhythm was continuous with the nighttime activity component as expressed under the prior LDLD entrainment conditions.

Discussion

The present study demonstrates that hamsters entrained to LD 14:10 will split their activity rhythms in response to a shortening of the nighttime scotophase when a second, afternoon scotophase is paired with that

Table 2 Correlation coefficients between parameters of nighttime and afternoon activity components for each of six animals exhibiting rapid splitting and two hamsters with delayed splitting

Hamster	Onset$_a$-Onset$_n$	Onset$_a$-Offset$_n$	α_a-α_n	Counts$_a$-Counts$_n$
Rapid splitters				
No. 21	–0.92***	–0.59*	–0.94***	–0.88***
No. 23	–0.83***	0.19	–0.85***	–0.81***
No. 25	–0.65**	–0.66**	–0.52*	–0.58*
No. 26	–0.88***	–0.10	–0.79***	–0.74**
No. 27	–0.82***	0.51*	–0.75***	–0.84***
No. 28	–0.74***	–0.39	–0.56*	–0.64**
Late splitters				
No. 29	–0.83***	0.39	–0.83***	–0.64**
No. 30	–0.77***	0.37	–0.61*	–0.08

*$P < 0.05$;
**$P < 0.01$;
***$P < 0.001$

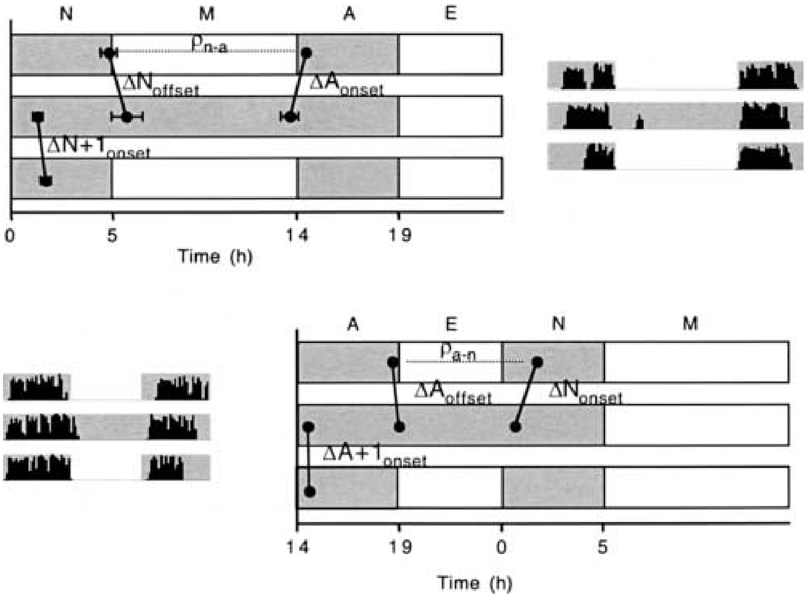

Fig. 6 Mean (±SEM) onsets and offsets of split activity components before, during and after omission of the morning (top) and evening (bottom) photophases. Each *line* represents a 24-h interval with successive days plotted below. Lines connect the same data measures collected on subsequent days. Error bars are contained within the symbol where not visible. To the *side* of each summary figure are representative 3-day actograms of single hamsters. In all panels, *shaded areas* indicate time of darkness

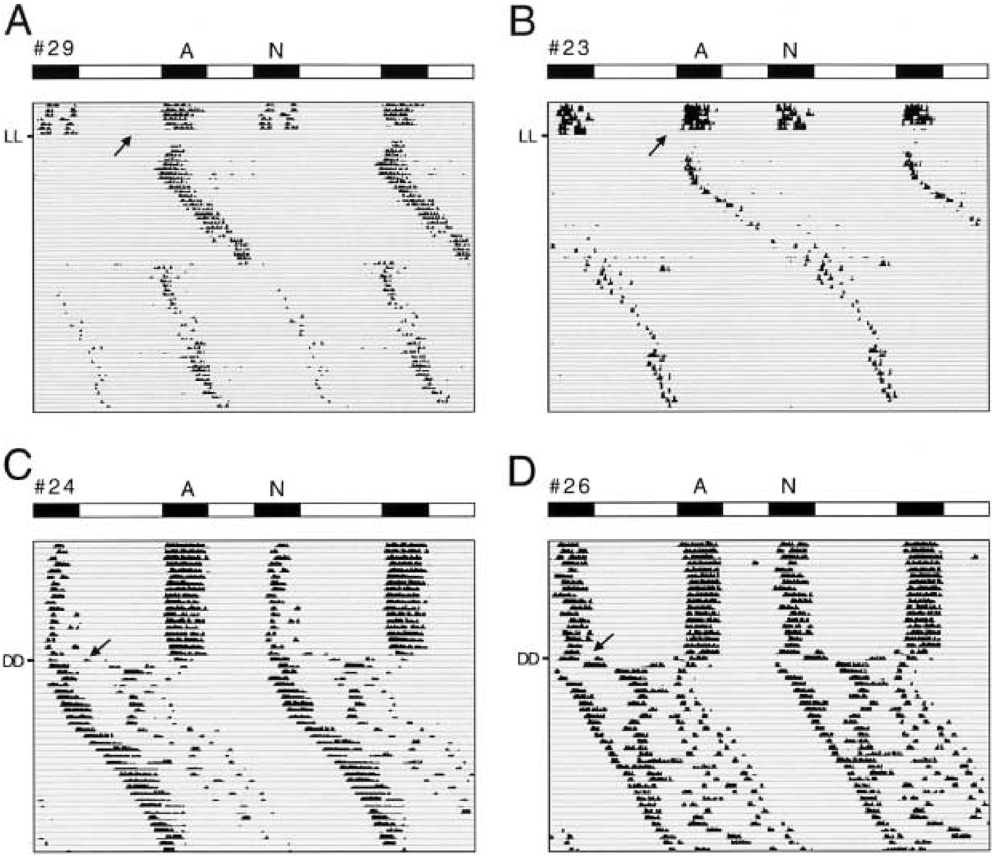

Fig. 7 Representative actograms of split hamsters transferred to constant light (**A**, **B**) or constant dark (**C**, **D**). Times of transition are noted on the actograms by *arrows* and on the left margin on actograms. Conventions as in Fig. 2

treatment. Splitting was not observed when the night was shortened without the second scotophase. The split rhythms that resulted emerged in a stereotypical fashion and remained entrained to an LDLD 9:5:5:5 photoperiod for the duration of the study but quickly rejoined in both DD and LL. This study demonstrates a third method – in addition to prolonged exposure to LL (Hoffmann 1971; Pickard et al. 1993; Pittendrigh 1967; Turek et al. 1982) and 7–17 days of daily NWR (Gorman and Lee 2001; Mrosovsky and Janik 1993) – sufficient to split hamster locomotor activity rhythms. Whether NWR-induced and photoperiod-induced split rhythms are identical in formal and physiological terms is not yet clear.

The entrained split rhythm represents two distinct subjective nights rather than a single long subjective night interrupted daily by one of the two photophases. Daily interruption of a long subjective night with a 5-h or 9-h light pulse would produce large daily phase shifts and thus be incompatible with stable entrainment patterns. Deletion of either the morning or evening light segment, moreover, induced no marked increase in activity and established that the absence of activity during these light phases is not attributable to negative masking. While the afternoon and nighttime components remained completely distinct with deletions of single photophases, in DD or LL they quickly rejoined. In earlier experiments, male Syrian hamsters also transiently expressed split rhythms in DD after a regimen of daily afternoon NWR and concomitant exposure to a LD cycle. Light-pulse probes further established that each split activity bout reflects a short, light-responsive subjective night flanked by light-refractory dead zones (Gorman et al. 2001). These NWR-induced rhythms remained split if hamsters were housed in LDLD 6:5:3:10 (Gorman and Lee 2001) or LDLD6:5:8:5 (M.R. Gorman, unpublished observations) rather than in DD. In both paradigms, therefore, the two photophases intervening between split components counteract the tendency of these two subjective nights to coalesce into one in DD.

The afternoon activity component appears to be principally entrained by delaying effects of light acting early in that component's subjective night. Whereas continuation of darkness into evening had no influence on offset of the afternoon activity component, deletion of the morning pulse tended to phase-advance the onset of the afternoon activity bout. The phase angle of entrainment suggests a similar reliance on morning rather than evening light as activity onset more closely corresponds with its light:dark transition than does activity offset. Finally, in DD the afternoon activity component showed advances over the several days until rejoining was complete. Together these results suggest an oscillator with a $\tau < 24$ h entrained by phase delays early in subjective night.

The formalisms of the nighttime component are less clear. Deletion of the evening light period advanced the onset of nighttime activity suggesting that evening light normally delays this component. Morning light omissions, however, tended to extend activity offset consistent with an advancing role of this light pulse. Since activity was symmetrically distributed throughout the scotophase, both evening and morning light may entrain this component. Finally, in DD this component generally delayed until rejoining occurred, although advances were also observed 2–4 days into DD. Manipulation across a range of entrainment conditions will be necessary to assess this component more thoroughly.

Why did splitting occur under LDLD conditions? In Syrian hamsters, treatments that greatly compress alpha – simulation of extremely long photoperiods with 1-h skeleton pulses and exposure to T-cycles – commonly induce phase jumps of activity into dark periods previously falling during the subjective day. In hamsters, the "minimum tolerable night" under skeleton photoperiods is around 6 h (Pittendrigh and Daan 1976a). While the 5-h night used in this experiment is below the minimum tolerated by Syrian hamsters, the full photoperiods experienced by control hamsters offered no alternative entrainment opportunity, and thus these hamsters entrained to the LD 19:5. An afternoon 5-h scotophase, which would have negligible effects on entrainment to a typical (e.g., 10–12 h) night, however, introduces the possibility of phase jumps. Complete inversion from nighttime to afternoon activity without splitting would not be expected to yield a more stable entrainment pattern as the two dark phases are the same length. However, if component oscillators of the circadian pacemaker are easily temporally dissociated, as is clear from other work with this strain (Gorman and Lee 2001), then some circadian components may be phase-shifted to express activity during the afternoon scotophase, just as occurs with NWR-induced split rhythms (Gorman and Lee 2001).

The analysis of the emergence of split rhythms lends support to a multi-oscillator view of the circadian pacemaker. In the present study, the stable split state was achieved progressively over several days as if successive cohorts of component oscillators were rephased from expressing activity in the nighttime scotophase to expressing it in the afternoon scotophase. This multi-oscillator view is contrasted with an exclusively dual-oscillator view in which one of two indivisible component oscillators is similarly phase-shifted. In the latter case, parameters of the nighttime activity component would not be expected to be correlated with those of the afternoon component once the split occurred. The data are contrary to this pattern in every instance. Split activity first appeared in the afternoon always near the end of the 5-h scotophase. Afternoon activity incrementally advanced as the bout grew longer and contained more total wheel revolutions. Correlated with these changes, the nighttime component began later and became shorter with fewer wheel revolutions. Importantly, the offset of the nighttime bout was not altered consistently, suggesting that afternoon activity components were mechanistically related to nighttime activity onset rather than offset. A multi-oscillatory view was also suggested by studies of NWR in which the onset of nighttime activity was delayed in proportion to the number of days of NWR. By titrating the number of days of NWR, some or all home-cage activity could be phase-shifted into the afternoon scotophase (Gorman and Lee 2001).

The splitting coincident with cage changing in two hamsters raises the possibility that darkness- or novelty-induced activity may mediate the photoperiodic induction of splitting. Mrosovsky and Janik (1993) and Gorman and Lee (2001) both demonstrated that NWR in mid-afternoon induced split activity rhythms in hamsters maintained in LD 14:10. In the former experiment, hamsters running less than 4000 rev/3 h did not show the same circadian reorganization. Uniformly high novelty-induced running levels, however, were obtained in the latter study. In two hamsters in the current study, splitting did not occur until cages were changed at which point robust running was observed as is typically seen after transfer to novel cages equipped with running wheels. In the same way that activity provoked by transfer to novel cages split rhythms in prior studies, running induced by the cage change may have precipitated the reorganization of circadian oscillators in these two hamsters. However, such a mechanism appears insufficient to account for splitting in the larger complement of splitting hamsters. Because hamsters remained in their home cages throughout this study, the actograms precisely depict the amount of afternoon running which is potentially mediating the splitting phenomenon. As seen in Figs. 2, 3, 4, and 5, the first afternoon activity components were short with total activity counts far from the threshold amounts cited by Mrosovsky and Janik (1993)as necessary for phase-shifting rhythms (242 ± 64 rev, 322 ± 47 rev for day 1 and day 2 of splitting, respectively, $n = 6$). The threshold amount of activity for circadian reorganization was not determined in the current study, and it is problematic to compare our results directly with those of other studies. However, in previous, the levels of afternoon activity required to induce splitting exceeded by an order of magnitude that which occurred on the first days of photoperiod-induced splitting reported here. This argues against a mediating role of activity in generating these splits.

The results in constant conditions indicate that either split activity component may become associated with the activity onset of the rejoined rhythm. In LL, the afternoon activity component became dominant and the nighttime component disappeared. In contrast, the nighttime activity component was associated with the activity onset of the fused rhythm expressed in DD. In earlier studies of LL-induced split rhythms, either activity component could become the activity onset of the fused rhythm, as long as it was associated with the LL to DD transition (Earnest and Turek 1982).

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