ELSEVIER

Physiology & Behavior 76 (2002) 469–478

PHYSIOLOGY
&
BEHAVIOR

Split circadian rhythms of female Syrian hamsters and their offspring

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Received 25 September 2001; received in revised form 13 February 2002; accepted 12 March 2002

Abstract

In several mammalian species, circadian pacemakers of breeding females synchronize the developing clocks of offspring by as of yet unspecified mechanisms. The present study assessed whether maternal communication of circadian rhythms extends beyond setting pacemaker phase to include transfer of a fundamental reorganization of component circadian oscillators from dams to pups. In Experiment 1, a regimen of daily novel wheelrunning previously demonstrated to split activity rhythms of adult male hamsters into two discrete components was shown to similarly reorganize female hamster rhythms. In Experiment 2, females split by this method and unsplit controls exposed to similar light environments were mated with males. Split and unsplit females were equally fecund, but the former weaned pups of lower body weight. After weaning into running wheel cages, offspring of split dams were more likely to exhibit split activity rhythms than were offspring of unsplit females. Among pups not categorized as split, moreover, maternal entrainment nonetheless influenced distribution of pup activity across the 24-h cycle. Entrainment patterns of split and unsplit pups resembled those of adults. Thus, split and unsplit hamster dams provide different entraining signals to their developing offspring. The influence of maternal rhythms extends beyond entraining phase to alter interactions between component circadian oscillators that underlie split activity bouts. Maternal effects did not persist beyond the second week postweaning in split or unsplit hamsters, however, and rhythms of many split pups later joined. Thus, the maternal influence on the pup's circadian pacemaker may be transient.

Keywords: Splitting; Circadian; Maternal communication; Photoperiod; Nonphotic; Wheelrunning; Novelty

1. Introduction

The suprachiasmatic nucleus (SCN) of the hypothalamus is the endogenous source of circadian rhythmicity in physiology and behavior of mammalian species [28]. Under constant environmental conditions, the rhythms generated by this structure have a period close to, but not exactly, 24 h. In the presence of a natural light/dark (L/D) cycle, the rhythms are entrained to match the 24-h day by a phase-dependent resetting action of light. Circadian rhythms and their resetting mechanisms presumably evolved to enable animals to anticipate the regularly changing environmental conditions of the day/night cycle.

Although overt rhythms in hamster behavior are not readily measured in the first weeks of postnatal life, entrained circadian oscillations of metabolic activity in the fetal SCN are evident prior to birth [22]. Because hamsters give birth to altricial young in burrows with minimal or no light exposure, there may be strong selection pressure for nonphotic entrainment mechanisms prior to burrow emergence. Indeed, in a variety of rodent species, maternal circadian rhythms entrain the rhythms of their offspring beginning prior to birth [24]. The mechanism by which the maternal circadian clock resets the clocks of her offspring is unclear, but appears to involve redundant daily cues. Mothers lacking circadian rhythms after SCN lesions produce pups exhibiting rhythms out of synchrony with those of littermates [5,23]. When exposed during pregnancy to daily scheduled food access [29], melatonin [6], or dopamine agonists [27], these dams all raise litters with synchronized circadian rhythms. A separate literature demonstrates that rodent mothers, via distinct mechanisms, also communicate to their pups information about the season of the year [12,15]. The present study was designed to explore whether rodent dams could transmit a novel and distinct dimension of circadian organization—the interaction of component circadian oscillators—to their pups, and if so, whether atypical maternal rhythms during the time of SCN cell differentiation would exert permanent organizational effects on circadian rhythms in adult offspring.

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0031-9384/02/$ – see front matter
PII: S0031-9384(02)00726-6

In adult hamsters and other mammalian species, prolonged exposure to constant light induces a temporal reorganization of circadian rhythms termed splitting [19,26]. Whereas hamsters in constant conditions typically alternately express daytime and nighttime behavior and physiological events with a circadian period, hamsters with split rhythms show a doubled frequency of the rest/activity cycle. The two activity bouts per circadian cycle are likely mediated by separate neural oscillators located within or between the two SCN [7,16,30]. Normally coupled to generate a unimodal rest/activity cycle under natural conditions, the coupling between oscillators is altered by constant light so that the oscillators assume an alternative temporal relationship, generating two activity bouts each 24 h with bouts roughly 12 h apart.

Constant light exposure used to split rhythms of adult mammals, however, is not conducive to reproduction in nocturnal rodents. An alternative method for splitting rhythms has been recently reported [10,18]. Briefly, in male hamsters housed in 14-h day/10-h night (LD14:10), timed daily exposure to novel wheelrunning (NWR) in the afternoon elicits marked changes in circadian organization. The onset of nocturnal wheelrunning behavior in the home cage is progressively delayed with each subsequent day of NWR. If daily transfer to novel wheels is discontinued when nocturnal onset has been delayed by 5 h and hamsters are then left in their home cages, males exhibit a split pattern of wheelrunning behavior in the home cage. One bout of activity occurs during the latter half of the 10-h night, whereas a second bout appears at a time corresponding to the previous exposures to NWR. This entrainment pattern may be maintained indefinitely with appropriate lighting schedules [10] (unpublished observations).

To determine the influence of split maternal rhythms on pups, we first assessed whether adult females, like males, could be induced to split their activity rhythms by exposure to NWR and whether such females were fecund. We then assessed whether pups born of mothers with split activity rhythms would be more likely to express split rhythms themselves. If so, we queried whether exposure to split maternal cues throughout development would exert organizational effects on circadian rhythms of adult offspring or whether any maternal effects would be transient. The influence of the maternal entrainment state on the rhythms of pups postweaning was assayed separately under postnatal conditions known in adults to (1) favor perpetuation of a split activity pattern or (2) permit recoupling of rhythms into the unsplit state [9,10].

2. Methods

2.1. Experimental procedures

2.1.1. Experiment 1

Female Syrian hamsters ($n = 17$, HsdHan:AURA; Harlan, Indianapolis, IN), 8–9 weeks of age, were singly housed in LD14:10 (lights off 1900 PST) with food (Purina Rodent Chow #5001, St. Louis, MO) and water available ad libitum. Hamsters were housed in polypropylene cages (27 × 20 × 15 cm) equipped with running wheels (17 cm in diameter) located in individual light-tight ventilated chambers with light intensity of ~150 lx at the bottom of the cage during the light phase and dim (< 1 lx) green illumination during the dark phase.

After entrainment to LD14:10, hamsters were transferred daily to another room and locked into unfamiliar Wahmann wheels (34 cm in diameter) 0–15 min before lights were extinguished at 1100 PST (ZT4). At 1600 PST (ZT9), the lights were turned on and hamsters were returned to home cages in the light over the next 15 min. Animals were assigned to different novel wheels on subsequent days. All hamsters received a minimum of 7 days of NWR. If activity onset in the home cage was delayed by more than 5 h, NWR was discontinued and hamsters remained at home in an LDLD6:5:3:10 cycle (see Fig. 1). This photoperiod combines the 5-h dark pulse previously in effect during NWR with the preexperiment LD14:10. For hamsters that did not delay nighttime activity by 5 h, NWR was discontinued after 14–18 days and hamsters thereafter also remained at home in LDLD6:5:3:10. All hamsters were monitored for 5 weeks following the onset of NWR.

2.1.2. Experiment 2

The same female Syrian hamsters ($n = 16$) employed in Experiment 1, now 32 weeks of age, were transferred to larger polypropylene cages (48 × 27 × 20 cm) equipped with Nalgene (34 cm in diameter) running wheels in the same LDLD6:5:3:10 light cycle. In this experiment, multiple cages were housed in large, ventilated cabinets with no illumination during dark periods.

While some hamsters remained split between experiments, rhythms of others had coalesced into the typical unsplit pattern. Five hamsters with unsplit rhythms, therefore, were exposed again to an identical regimen of NWR as described for Experiment 1. The remaining hamsters with rejoined rhythms were not exposed to NWR and served as unsplit controls. One week following the completion of NWR, all 16 females were paired with unsplit stud males 1 h before onset of the afternoon dark period. Females were transferred from their home cage to that of a male, and the pairs were observed to determine if the female exhibited lordosis. Those showing lordosis remained with males for one additional hour before they were returned to their home cage. Nonreceptive females were returned to their cages before dark onset. Hamsters that failed to exhibit lordosis on six consecutive afternoons were then paired with males 1 h prior to the onset of the evening dark period until mating occurred. Females remained relatively undisturbed throughout gestation. Beginning 16 days after copulation, cages were examined for new litters, and the number of pups on the day of birth was counted.

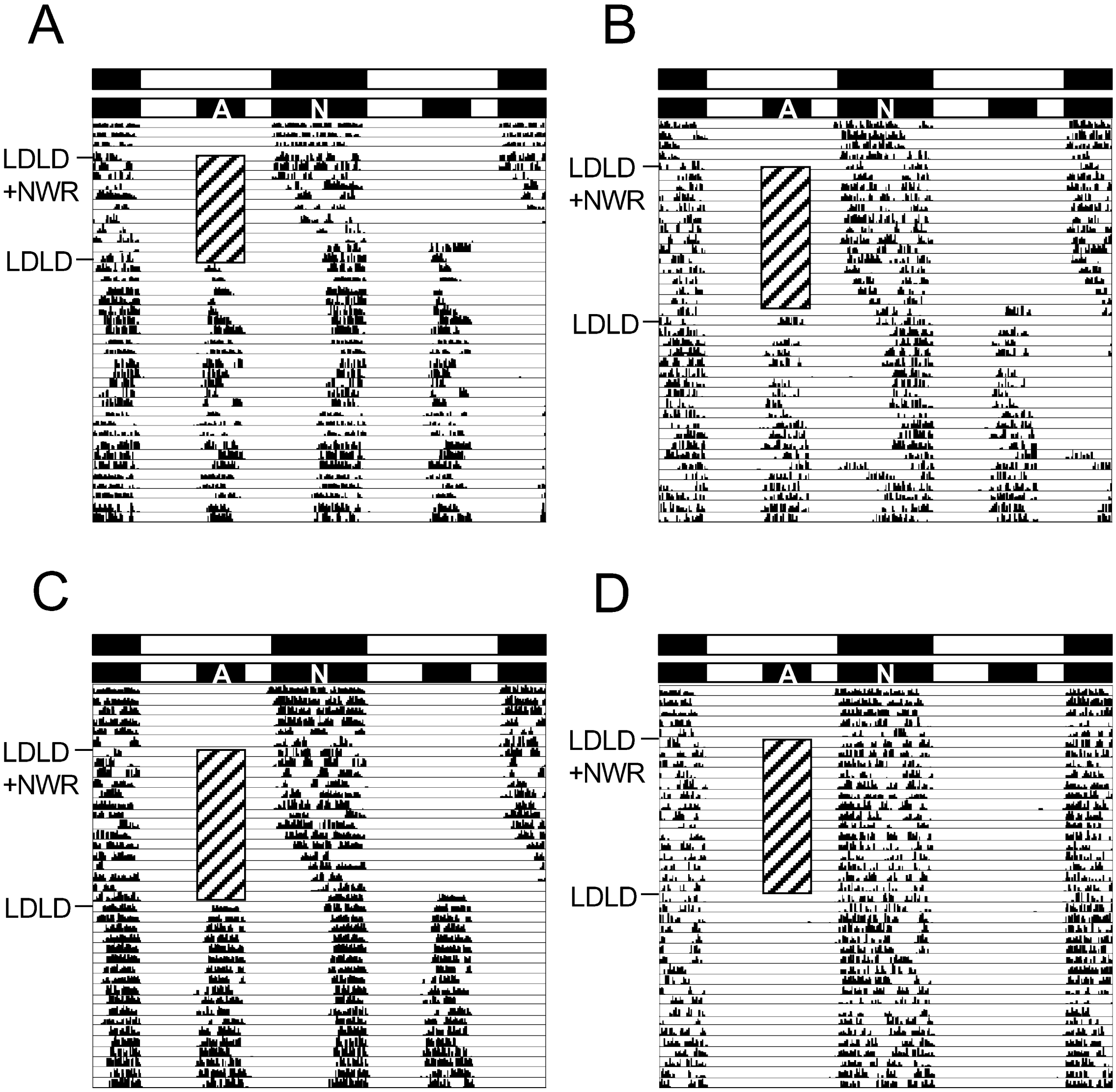

Fig. 1. Representative double-plotted actograms of adult female Syrian hamsters exposed in Experiment 1 to daily scheduled NWR. Data are unfiltered and are scaled on the ordinate from zero to the maximum number of revolutions for that hamster. Shaded rectangles above actograms depict initial photoperiod conditions (upper rectangles) and those in effect during and after NWR (rectangles immediately below). Shaded and open boxes represent times of dark and light, respectively, with nighttime and afternoon scotophases designated as N and A, respectively. Hatched boxes within actograms (left side) represent times of NWR exposure. Hamsters previously entrained to LD14:10 were transferred daily to dark novel wheels from ZT4–ZT9 (marked NWR + LDLD). After 7–18 days, hamsters remained at home in the same LDLD conditions. Hamsters in (A–C) exhibited progressive delays of nighttime activity in the home cage. Home cage rhythms were split into two components associated with each dark period following NWR. In (D), the home cage activity rhythm did not delay nor did a split rhythm appear in LDLD in the home cage.

At 20 days of age, pups from each litter were sexed, weighed and weaned into polypropylene cages (48 × 27 × 20 cm) equipped with stainless steel running wheels (17 cm in diameter) with multiple cages housed in ventilated cabinets. Pups from each litter were divided between two lighting conditions: LDLD6:5:3:10 (replicating that in which dams were previously housed) and LDLD6:5:8:5 (see Fig. 3). The latter photoperiod reflected a reduction of the previous 10-h nighttime dark period to 5 h. These lighting conditions remained in effect until the end of the experiment 6 weeks later.

2.2. Data collection and analysis

Running wheels were equipped with switches triggering electrical closures that were recorded and compiled into 10-min bins by Dataquest III or VitalView software (Mini-Mitter, SunRiver, OR). In both experiments, home cage wheelrunning rhythms were assessed with actograms prepared with Actiview software. Additionally, in Experiment 1, intensity of running in novel wheels was quantified by summing the number of wheel revolutions in each 5-h exposure to NWR.

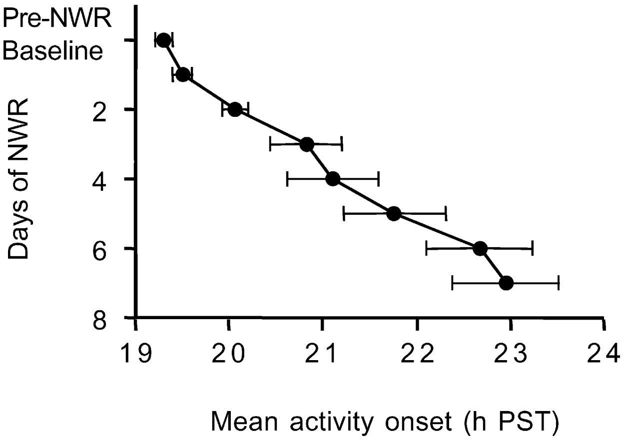

Fig. 2. Mean (±S.E.M.) nighttime activity onset of hamsters ($n = 14$) responding to daily scheduled NWR with reorganization of circadian activity. Home cage activity onset was determined prior to NWR (baseline) and following each of the first 7 days of NWR (*Y*-axis).

For adult hamsters of Experiment 1, home cage activity onset was defined as the first point in the night in which activity levels exceeded 50 counts/10-min bin. Daily phase shifts in home cage activity onset, calculated by comparing values before and after each day of NWR, were assessed for correlations with the number of revolutions run in the novel wheels. Phase shifts greater than 90 min were excluded from correlation analysis because they typically reflected artifactually suppressed running (e.g., due to a blocked wheel) rather than stable phase shifts.

In Experiment 2, activity rhythms of pups were analyzed quantitatively over three separate intervals postweaning: Days 3–6, 7–14 and 35–42 (Week 6). The number of wheel revolutions in each 10-min bin was averaged to produce a 24-h histogram for each hamster over each analysis interval. Because activity levels are low in juveniles and increase as hamsters mature, no absolute threshold for activity levels could be established. Instead, activity was identified as a bout for further analysis if two consecutive bins in the 4- or 8-day histogram contained activity levels exceeding the overall daily mean activity. A pup's activity rhythm was considered split if each dark period contained activity meeting this criterion. For a noncategorical measure of distribution of pup activity, the percent of daily activity from 1 h before to 1 h after the afternoon dark period (1000–1700 PST) was calculated, hereafter referred to as "percent afternoon activity."

For every activity component (whether present in one or both dark periods), activity onset was defined as the first 10-min histogram bin exceeding threshold amounts of activity and followed within 20 min by a second interval likewise exceeding threshold. Activity offset was the last time point above threshold which was immediately preceded by a bin exceeding threshold. For each activity component, activity duration (α) was defined as the time difference between activity onset and offset. Activity onsets and offsets are sometimes expressed as phase angles of entrainment in relation to the associated L/D or D/L transition ($\Psi_{\text{onset, L/D}}$ or $\Psi_{\text{offset, D/L}}$). Identical algorithms were applied to activity data of adult females over the 4 days preceding copulation with males.

Effects of maternal entrainment state and postweaning photoperiod on the incidence of splitting were assessed with contingency statistics (chi-square; Statview 5.0, SAS Institute, Cary, NC). Continuously varying measures of activity were analyzed using analysis of variance (ANOVA). Because split and unsplit rhythms represent categorically distinct (noncontinuously varying) entrainment patterns, split pups and unsplit pups were separated for these entrainment analyses. Because violations of ANOVA assumptions are difficult to detect with small sample sizes, all effects were corroborated by nonparametric Mann–Whitney *U* tests (not reported).

Differences in reproductive performance of split and unsplit dams were evaluated with the litter as the unit of analysis. Litter size (number of pups counted on day of birth), birth-to-weaning mortality per litter, sex ratio at weaning (number of males/total pups) and average litter pup weight at weaning were assessed by ANOVA (Statview 5.0; SAS Institute).

3. Results

3.1. Experiment 1

Hamsters ran robustly in the novel wheels (mean: 5251 ± 417 revolutions/5 h; range: 1285–7075 revolutions/5 h). Nearly all hamsters (14/17) exhibited a marked reorganization of locomotor activity in the home cage during and following NWR (Fig. 1A–C). Typically, nocturnal activity onset was progressively delayed with subsequent days of NWR (Figs. 1 and 2). Following discontinuation of NWR, home cage wheelrunning activity was divided between the 5-h afternoon dark period and the latter half of the 10-h nighttime dark period. This pattern persisted in the weeks following the cessation of

Table 1
Mean (±S.E.M.) entrainment parameters of split and unsplit females in LDLD6:5:3:10 during the 4 days prior to pairing

	Split females			Unsplit females	
	Nighttime	Afternoon	*n*	Nighttime	*n*
$\Psi_{\text{onset, L/D}}$ (h)	− 4.13 ± 0.57*	− 1.06 ± 0.26	12	− 0.17 ± 0.07	4
$\Psi_{\text{offset, D/L}}$ (h)	1.35 ± 0.40	0.26 ± 0.13	12	2.25 ± 0.60	4
α (h)	4.53 ± 0.49 *	3.68 ± 0.29 *	12	7.58 ± 0.57	4
Percent afternoon activity	37.9 ± 3.6 *		12	0.8 ± 0.4	4
Total daily activity	29511 ± 2290		12	27539 ± 1240	4

* Differs significantly from nighttime values of unsplit females ($P < .05$).

Table 2
Effects of maternal entrainment state on litter characteristics (mean ± S.E.M.)

	Split dams	Unsplit dams	P
Pups/litter	4.43 ± 1.02	5.50 ± 0.65	
Sex ratio	0.43 ± 0.09	0.40 ± 0.05	
Pup mortality	1.14 ± 0.55	0.50 ± 0.50	
Weaning pup weight (g)	15.5 ± 0.5	21.7 ± 3.6	$<.05^{a}$
Proportion of litters with split pups	7/7	2/4	$<.05^{b}$

[a] ANOVA.

[b] Chi-square.

NWR. Three hamsters failing to exhibit this reorganization of circadian activity ran substantially less in the novel wheels than did the complement of hamsters (2435 ± 1036 versus 5854 ± 257 revolutions/5 h; $P<.05$, Mann–Whitney U test). In these hamsters, nocturnal activity onset was not markedly delayed (Fig. 1D). Fig. 2 illustrates the mean effect of each day of NWR on nocturnal activity onset among the 14 hamsters responding to this treatment.

Not all hamsters with reorganized circadian activity responded simultaneously to the NWR. Some animals ran robustly on the very first days of NWR and showed concomitant delays in nocturnal activity onsets (Fig. 1A). Others ran at high rates and phase-delayed nighttime activity onset only after several days of NWR (Fig. 1B and C). The magnitude of the daily phase shift of nighttime activity onset was significantly correlated with intensity of activity in the prior episode of NWR ($r=0.412$, $df=155$; $P<.001$).

3.2. Experiment 2

In the five hamsters treated, scheduled NWR induced changes in circadian organization qualitatively similar to those reported in Experiment 1 (data not shown). Except in one instance, split rhythms were maintained throughout

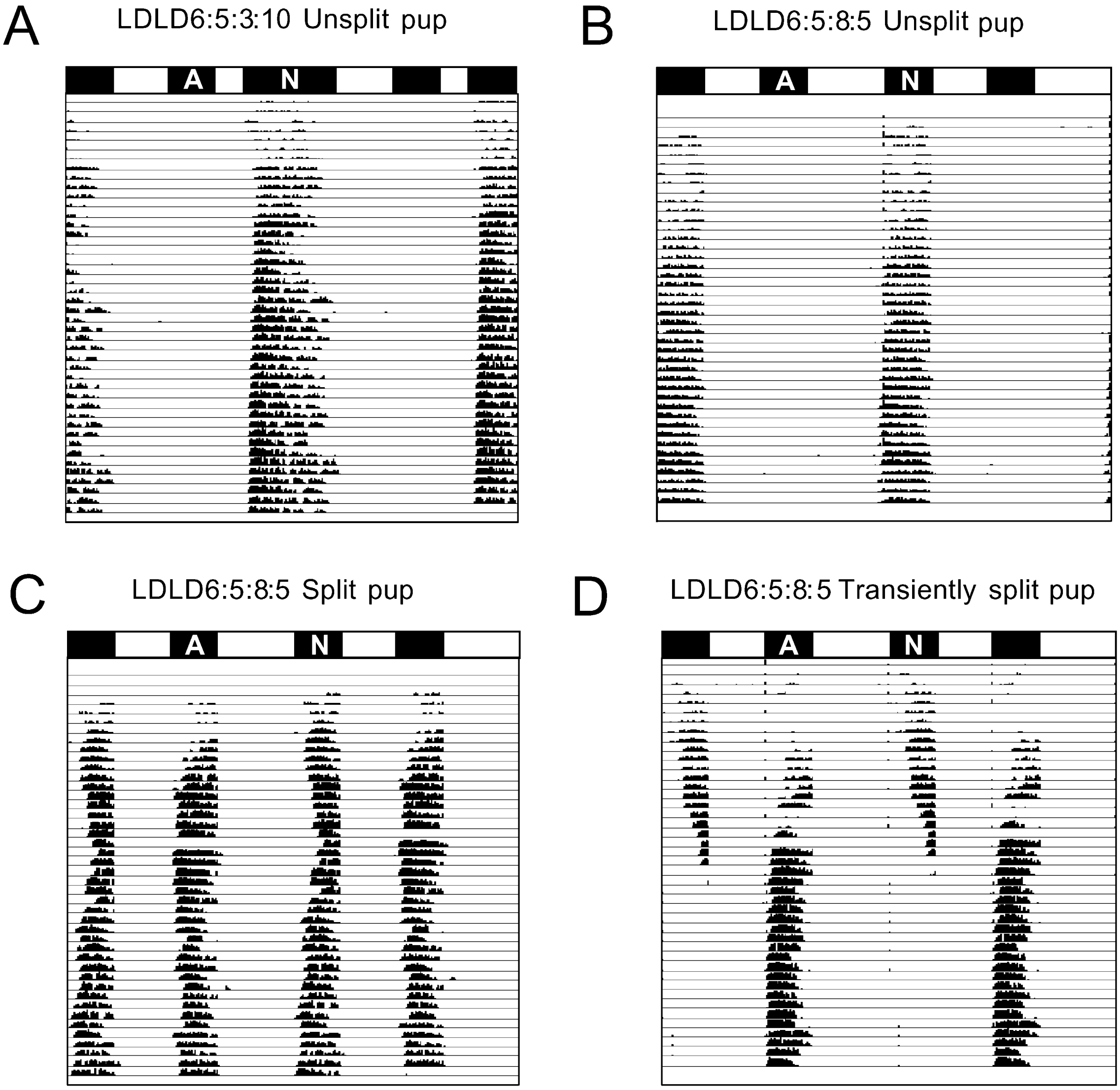

Fig. 3. Representative actograms of pups from time of weaning into LDLD6:5:3:10 (A) or LDLD6:5:8:5 (B–D). Conventions as in Fig. 1.

gestation. The one female that lost her split was reclassified as unsplit because rejoining occurred before the second half of pregnancy. In the 4 days prior to breeding, females classified as split exhibited two distinct daily bouts of activity (Table 1) with entrainment parameters qualitatively similar to those previously reported for males [9,10]. Mean nighttime activity onset occurred several hours later after the L/D transition in split compared to unsplit dams [Table 1; $F(1,14)=15.2$; $P<.01$; negative values indicate phase lags of activity onset to the L/D transition]. Activity offsets anticipated the D/L transition, and $\Psi_{\text{offset, D/L}}$ did not differ between groups (Table 1). Correspondingly, α was significantly shorter among split dams [$F(1,14)=11.1$; $P<.01$]. A larger fraction of daily activity also occurred during the afternoon in split versus unsplit dams [$F(1,14)=33.3$; $P<.001$], although total activity did not differ between groups.

All 16 females copulated and 11 of these produced litters (7 of 11 split dams; 4 of 5 unsplit dams). Litters of split and unsplit dams did not differ in the number of pups per litter, sex ratio or postnatal mortality (Table 2). Pup weight at weaning, however, was significantly lower for litters born of split mothers ($P<.05$, ANOVA; $P<.10$, Mann–Whitney U test).

Pup wheelrunning activity rhythms showed varied patterns as depicted in Fig. 3. In general, pups ran least during the first week of recording but reached mature running levels shortly thereafter. In one common pattern (Fig. 3A and B), activity was largely restricted to the nighttime dark period. In another (Fig. 3C), activity was consistently divided (i.e., split) between the nighttime and afternoon dark periods. In a third pattern, activity was transiently split (Fig. 3D); a split rhythm was manifest early in the record but was later lost as the two activity components integrated into a single bout in the afternoon dark period.

3.2.1. Incidence of pup splitting

Treated as independent data points and without regard to postweaning photoperiod, pups with split rhythms were more likely to be born of dams with split activity than of dams with unsplit rhythms: 14 of 18 split pups and 16 of 34 pups that never split were born to split mothers [$\chi^2(1)=4.5$; $P<.05$]. With the litter/dam as the unit of statistical analysis, split mothers more often produced litters containing one or more split pups than did unsplit mothers [Table 2; $\chi^2(1)=4.3$; $P<.05$].

Postweaning photoperiod also influenced the incidence of splitting among offspring (Table 3). In LDLD6:5:3:10, pups nearly universally exhibited unsplit rhythms, whereas significantly more splitting occurred in LDLD6:5:8:5 [$\chi^2(1)=7.9$; $P<.01$].

3.2.2. Entrainment parameters of split and unsplit pups

In LDLD6:5:3:10, only three pups met the criterion for splitting during any analysis interval, and did so only during Days 3–6 (Table 3). As would be expected, these animals expressed a greater percentage of daily activity in the afternoon of Days 3–6 compared to unsplit pups [Table 3; $F(1,18)=4.8$; $P<.05$]. The nighttime activity of these three split pups, however, did not differ from that of unsplit pups in onset or duration (data not shown). An effect of maternal entrainment status could not be assessed among split pups as there were only three and all were born of split mothers.

Table 3
Incidence of pup splitting as a function of postweaning lighting conditions and percentage (mean ± S.E.M.) of daily activity associated with the afternoon dark period (from 1 h before to 1 h after)

	Splitting incidence			Percent afternoon activity		
	Days 3–6[a]	Days 7–14	Week 6	Days 3–6	Days 7–14	Week 6
Postweaning LDLD6:5:3:10						
Split pups	3	0	0	8.6 ± 2.1		
Unsplit pups	17	23	23	3.9 ± 0.8	0.4 ± 0.1	0.3 ± 0.1
P				< .05		
Postweaning LDLD6:5:8:5						
Split pups	13	8	4	29.1 ± 5.1	54 ± 7.5	59 ± 11.0
Unsplit pups	13	21	26	2.9 ± 0.8	0.6 ± 0.2	0.8 ± 0.2
P				< .001	< .001	< .001

Sample size corresponds to incidence table (left), except that six unsplit hamsters in LD6:5:8:5 with exclusively afternoon activity are excluded from Week 6 percent afternoon activity means.

[a] Numbers are reduced due to lack of running in some hamsters.

Among pups classified as unsplit in LDLD6:5:3:10 ($n=17$), no influence of maternal entrainment state was found for various measures of nighttime activity during Days 3–6: onset, offset and α did not significantly differ among pups of split versus unsplit dams (Table 4). Nevertheless, a significantly greater percentage of daily activity occurred in the afternoons of Days 3–6 among unsplit pups of split versus unsplit mothers [Table 4; $F(1,15)=5.2$; $P<.05$]. At later time points, neither percent of activity around the afternoon dark period nor any other measure of activity rhythms differed between unsplit pups of split versus unsplit mothers (data not shown).

Mean entrainment patterns of split and unsplit pups weaned into LDLD6:5:8:5 are shown for all intervals in Fig. 4. During Days 3–6, differences in neither nighttime activity onset nor nighttime activity offset reached statistical significance [$F(1,24)=3.4$ and $F(1,24)=3.5$ for onset and offset, respectively; $.05>P>.10$ for both], but nighttime α was significantly greater for unsplit pups than for split pups [Fig. 4A; $F(1,24)=5.1$; $P<.05$]. As would be expected, a greater percentage of activity occurred in the afternoon period among split hamsters [Table 3; $F(1,24)=26.0$; $P<.001$].

During Days 7–14, five hamsters previously classified as split exhibited a single integrated activity profile confined to the evening dark period. During this interval, the remaining split pups exhibited significantly later nighttime activity onsets [$F(1,27)=46.7$; $P<.001$] and shorter α values [$F(1,27)=39.1$; $P<.001$] than did unsplit hamsters (Fig. 4B). They also expressed a greater percentage of activity in

Table 4
Influence of maternal entrainment state on entrainment parameters (mean ± S.E.M.) of split and unsplit pup rhythms during Days 3–6

	Nighttime component				
Unsplit pups	Onset (h PST)	Offset (h PST)	α (h)	Percent afternoon activity	*n*
LDLD6:5:3:10					
From split dams	19.35 ± 0.49	3.37 ± 0.39	8.12 ± 0.80	5.3 ± 1.2	10
From unsplit dams	19.41 ± 0.24	4.00 ± 0.47	8.60 ± 0.51	1.9 ± 0.3	7
P				< .05	
LDLD6:5:8:5					
From split dams	21.67 ± 0.89	5.20 ± 0.06	7.53 ± 0.93	3.8 ± 1.3	5
From unsplit dams	23.65 ± 0.56	4.90 ± 0.10	5.25 ± 0.50	2.4 ± 1.0	8
P	< .10	< .10	< .05		

	Nighttime component					Afternoon component		
Split pups	Onset (h PST)	Offset (h PST)	α (h)	Percent afternoon activity	*n*	Onset (h PST)	Offset (h PST)	α (h)
LDLD6:5:8:5								
From split dams	0.74 ± 0.4	4.87 ± 0.10	4.35 ± 0.40	29.4 ± 6.3	9	12.98 ± 0.24	15.02 ± 0.42	2.04 ± 0.40
From unsplit dams	22.79 ± 0.8	3.71 ± 0.69	4.92 ± 1.23	28.3 ± 9.6	4	11.46 ± 0.63	14.25 ± 0.66	2.79 ± 1.27
P	< .05	< .05				< .05		

Times are represented as decimal clock hours (0–24 PST).

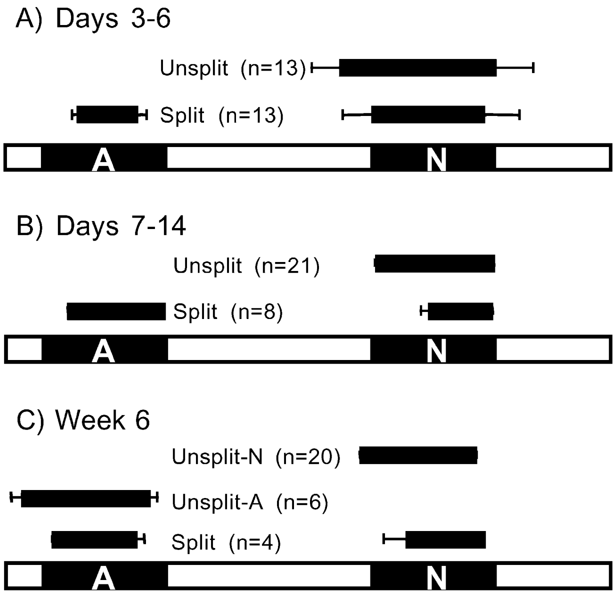

Fig. 4. Activity profiles of split and unsplit hamster pups on LDLD6:5:8:5 during Days 3–6 (A), Days 7–14 (B) and Week 6 (C). Mean (± S.E.M.) activity onset and activity offset are indicated by left and right margin of black bars, respectively. Bar length represents activity duration (α). Lighting conditions are displayed below with black rectangles indicating times of darkness. In (C), unsplit pups with activity concentrated in afternoon and nighttime dark periods are depicted separately. In some cases, errors bars are not discernible.

the afternoon compared to unsplit hamsters [Table 3; $F(1,27) = 143.1$; $P < .001$].

In the sixth week of LDLD6:5:8:5, six previously split pups consolidated activity into an unsplit state with activity concentrated in the afternoon dark period. Two additional pups, unsplit prior to Day 14, exhibited split rhythms at Week 6. As occurred earlier, the nighttime activity onset was later [$F(1,22) = 21.4$; $P < .001$] and nighttime α was shorter [$F(1,22) = 10.0$; $P < .01$] for split hamsters than for unsplit hamsters with activity concentrated in the nighttime dark period (Fig. 4C). Comparison of afternoon activity of split hamsters versus that of unsplit hamsters expressing activity in the afternoon dark period yielded similar results: afternoon activity onset was marginally later [$F(1,8) = 3.7$; $P < .10$] and α shorter [$F(1,8) = 7.6$; $P < .05$] for split hamsters.

A maternal influence on pup activity rhythms in LDLD6:5:8:5 was apparent among pups classified as unsplit during Days 3–6 (Table 4). Those from split mothers exhibited longer α values than did those from unsplit mothers [$F(1,11) = 5.6$; $P < .05$]. Activity onset occurred marginally earlier and offset marginally later [$F(1,11) = 4.0$ and $F(1,11) = 4.6$, respectively; $.05 < P < .10$ for both] among pups from split versus unsplit dams. No differences between cohorts appeared during Days 7–14 or Week 6 (data not shown).

Maternal influences on Days 3–6 activity were also apparent in the group of pups classified as split in LDLD6:5:8:5 (Table 4). Among this cohort, pups with split mothers initiated [$F(1,11) = 6.6$; $P < .05$] and terminated [$F(1,11) = 6.4$; $P < .05$] nighttime activity later than did pups from unsplit mothers. Afternoon activity onset occurred later in split pups with split dams compared to those with unsplit mothers [$F(1,11) = 7.9$; $P < .05$]. No maternal influence was discernible in later analysis intervals (data not shown).

4. Discussion

Scheduled NWR induced marked reorganization of circadian rhythms of adult female Syrian hamsters, splitting activity rhythms into two components that were entrained, respectively, to the two dark periods of an LDLD photocycle. Female hamsters with split rhythms were sexually receptive shortly before the afternoon dark period and produced viable litters at rates comparable to those of unsplit females. Pups born of split dams were themselves more likely to split their activity between the two dark periods in LDLD6:5:8:5 during Days 3–6. Among pups not classified as split, maternal entrainment state nevertheless influenced distribution of locomotor activity in reference to the LDLD cycle. As minimal splitting occurred in LDLD6:5:3:10, both maternal condition and the postweaning light schedule influence the incidence of splitting in juvenile hamsters. These results extend the known influence of maternal circadian rhythms on pup circadian phase to a novel domain.

Several fundamental parameters of hamster circadian rhythms, including the propensity to split after prolonged exposure to constant light, are known to be sexually dimorphic [3,13,17]. The response of adult females to scheduled daily NWR, in contrast, was qualitatively similar to that previously observed in males [10], although minor methodological differences between the current and previous experiments preclude a quantitative conclusion about sex differences. As in males, each day of intense NWR induced a delay of nighttime wheelrunning activity in the home cage [10,18]. As was also the case with males [10], split rhythms appeared when the afternoon dark period associated with NWR was continued in the home cage environment to yield an LDLD6:5:3:10 photocycle. In males, transfer of split hamsters to constant dark (DD) inevitably induces a rapid recoupling of rhythms into the typical unsplit state [10]. Because males [10] (Gorman, unpublished observations) and females (current experiment) may maintain these split rhythms indefinitely under LDLD6:5:3:10, the rhythms may be considered entrained. A number of females in Experiment 1, however, reintegrated their activity patterns into an unsplit state with activity confined to the 10-h dark period. This result supports an earlier conclusion that split and unsplit rhythms reflect alternative stable entrainment states of the circadian pacemaker [9–11].

In Experiment 2, pups exhibited entrainment patterns similar to those previously reported for adults [9,10]. In addition to generating two activity bouts daily, split offspring entrained to LDLD6:5:8:5 with shorter nighttime α values and later nighttime activity onsets than did unsplit hamsters. While splitting in adult hamsters is known to reflect a temporal rephasing of component oscillators within the SCN [11], the same is not necessarily the case in pups. The afternoon activity of split pups could conceivably be mediated by non-SCN mechanisms recruited in response to maternal cues. This type of mechanism, however, would not predict altered nighttime activity for split pups. As nighttime activity is assuredly mediated by the SCN, we suggest that split rhythms in pups also reflect a temporal decoupling of circadian pacemakers within the SCN into two functionally distinct oscillators and that each of these oscillators mediates one activity bout.

Differences in rhythms of pups born of split versus unsplit mothers confirm and extend the known influence of maternal rhythms on development of pup circadian rhythms [2,21]. The altered incidence of splitting among pups born to split versus unsplit mothers establishes that the distribution of activity, like circadian phase, is transmitted transgenerationally. The strength of this effect was likely underestimated by the criteria used to identify split rhythms, as even pups classified as unsplit expressed greater activity in the afternoon if they were born to split mothers. Whereas this subthreshold splitting appeared in both photoperiods, only in LDLD6:5:8:5 did the maternal entrainment state predict splitting in pups. Thus, splitting in juvenile hamsters likely reflects joint actions of maternal and environmental light cues. In setting of pacemaker phase among unsplit Syrian hamsters, the strength of maternal entrainment cues diminishes postnatally [4]. Entrainment by light, in contrast, is possible only after innervation of the SCN by the retinohypothalamic tract in the second week of life [14]. If maternal cues to induce or maintain split rhythms in their pups are diminished postnatally, then pups may reintegrate their rhythms unless precluded from doing so by entraining LDLD cycles. Exposure to LDLD6:5:8:5 postweaning may arrest this reintegration and facilitate identification of an earlier maternal effect. LDLD6:5:3:10, in contrast, may permit reintegration and thereby obscure earlier maternal effects.

In contrast to clear maternal effects on the distribution of pup activity between the two dark phases, we found limited evidence for maternal effects on circadian phase within the split and unsplit category designations. Because split dams delay nocturnal activity onset by several hours relative to unsplit mothers, we anticipated that activity onset of unsplit pups would be delayed to match the delayed activity of a split mother. To our surprise, we found no effect of maternal entrainment state on nighttime activity onset of unsplit pups in LDLD6:5:3:10. For unsplit pups to mirror the nighttime activity onset of their split mothers, however, would have resulted in a marked compression of pup activity into the latter half of the 10-h nighttime dark period. In adult hamsters, such compression of α appears to reflect an unstable entrainment state [9,20]. In LDLD6:5:3:10, entrainment cues from the split dam may be insufficient to entrain a pup to this unstable configuration, causing the unsplit pup to instead entrain, as adult hamsters often do, with activity beginning shortly after onset of the dark period.

Maternal entrainment effects were discernible, however, among the class of unsplit hamsters maintained in LDLD6:5:8:5 postweaning. Unsplit pups of split mothers began activity markedly EARLIER (2 h prior to lights off) and had LONGER evening activity bouts during Days 3–6 than did unsplit pups of unsplit mothers. These findings were unexpected because split mothers themselves exhibited SHORTER evening activity bouts than did unsplit dams. The longer activity bouts of unsplit pups born of split mothers might be explained if these offspring were split previously and were undergoing recoupling during Days 3–6: incomplete rephasing of activity components into the evening dark period might resemble a long activity profile.

A split maternal rhythm was not necessary for splitting to emerge in juveniles weaned into LDLD6:5:8:5. As splitting was never observed among pups of unsplit dams in LDLD6:5:3:10, however, the former lighting condition appears to be a sufficient stimulus to induce splitting in some cases. The sufficiency of LDLD cycles to induce splitting has recently been demonstrated in adult male hamsters after transfer from LD14:10 to several different LDLD cycles containing two 5-h dark periods [9] (Elliott and Gorman, unpublished observations). A similar process would appear to operate in our juvenile hamsters, albeit at a low frequency. As entrainment patterns differed between split pups of unsplit and split dams, the mechanisms generating these splits may be distinct.

The processes by which dams influence pup splitting are as of yet undefined but may include genetic, hormonal or behavioral mechanisms. The latter two influences may occur either prenatally or postnatally. Studies of maternal entrainment of normal unsplit rhythms have established a role for both prenatal and postnatal cues. In cross-fostering studies, maternal cues in late gestation overrode discrepant postnatal signals in some litters. In other litters, postnatal cues exerted additional effects on litter synchrony but never completely overrode prenatal cues [4]. Analogous cross-fostering studies could untangle the relative importance of prenatal and postnatal cues in triggering split rhythms, as the present design unfortunately confounds these cues. In either case, the time course of maternal influence suggests a transient rather than an enduring organizational effect on the pacemaker of the offspring. Influences of maternal entrainment state within the split and unsplit classifications did not persist into the second week postweaning, and most split rhythms rejoined prior to the end of the study. Thus, split maternal rhythms beginning prior to the period of SCN differentiation, while able to exert short-term effects on pup rhythmicity, do not appear to permanently organize the circadian system of adult offspring.

In contrast to unsplit adult females who are not sexually receptive during the subjective day, split females mated readily when paired with males in the afternoon. As with many mammalian species, the surge of luteinizing hormone (LH) which induces ovulation and ovarian secretion of gonadal hormones, is under the control of circadian mechanisms [1,8]. The shift in timing of behavioral estrus, itself dependent on elevated titers of gonadal hormones, suggests that these endocrine events were either split or phase-shifted in females expressing split activity rhythms. The former interpretation is supported by findings from LL-induced splitting in which each of two daily activity bouts was associated with a surge in LH secretion, albeit of lower magnitude than that which occurs in unsplit hamsters [25]. Given the long interval (~18 h) of behavioral estrus in the hamster, however, phase advances of the LH surge into the afternoon or smaller phase delays later in the night would likewise predict afternoon lordosis. Further studies will be required to resolve this issue.

Acknowledgments

This work was supported by NIH grant HD-36460 and NSF grant IBN-9985676 and the UCSD Academic Senate Committee on Research. We are grateful to Nikki Zamora and Vincent Hsieh for excellent technical assistance, to Cesar Ramirez and Luis Escobedo for animal care and to Jeff Elliott for comments on an earlier draft of this manuscript.

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