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Enhancement and Suppression of Ultradian and Circadian Rhythms across the Female Hamster Reproductive Cycle

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Abstract

The impact of ovarian hormones on hamster ultradian rhythms (URs) is unknown. We concurrently monitored URs and circadian rhythms (CRs) of home cage locomotor activity during the estrous cycle, pregnancy, and lactation of Syrian hamsters. URs with a mean period of 4–5 h were evident during the dark phase in more than 90% of females on days 1 and 2 of the estrous cycle but were significantly less prevalent on cycle days 3 and 4. The period of the UR did not vary as a function of estrous cycle stage, but at all stages, the UR period was longer in the dark than the light phase. The UR acrophase occurred significantly earlier on cycle day 4 than on days 1 and 2, and UR robustness and amplitude were reduced on days 3 and 4. Robustness, mesor, and amplitude of CRs were greater during cycle days 3 and 4; timing of the CR acrophase was delayed on day 4 relative to all other cycle days. Effects of the estrous cycle on URs were evident only during the dark phase. The proportion of hamsters displaying dark phase URs increased significantly during early and late gestation and decreased during lactation. Pregnancy significantly increased UR complexity, robustness, and amplitude. The emergence of URs over gestation was paralleled by decrements in the robustness and amplitude of CRs, which also were absent in a significant proportion of dams during lactation but re-emerged at weaning of litters. The changing endocrine profile of the estrous cycle, hormonal dynamics of pregnancy and lactation, and nursing demands placed on dams are each associated with alterations in the expression of ultradian and circadian locomotor rhythms. Diminution of CRs and augmentation of URs may afford greater behavioral flexibility during life stages when interactions with mates and offspring are less predictable.

Keywords

locomotor activity; gonadal steroids; estrous cycle; pregnancy; lactation

Ultradian rhythms (URs) are evident at every level of biological organization. Despite claims that URs are “the primary rhythmic signature of the living state” (Yates and Yates,

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CONFLICT OF INTEREST STATEMENT

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NOTE

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2008) and that “they can be considered biological structures that may have adaptive value with regard to the internal temporal order for organisms” (Scannapieco et al., 2009), virtually nothing is known about the neuroendocrine substrates that control URs.

Generation of URs is not contingent on a functional circadian system. URs persist despite loss of circadian organization effected by exposure of rats to constant light (Honma and Hiroshige, 1978) or by destruction of the suprachiasmatic nucleus (SCN) in rats (Eastman et al., 1984), Syrian hamsters (Rusak, 1977; Refinetti, 1994), or common voles (Gerkema et al., 1990). The circadian clock is not, however, without effects on the ultradian system. An increased number of significant ultradian periodicities was positively associated with the power of the hamster free-running circadian rhythm (Refinetti, 1994), and hamsters bearing the *tau* mutation had shorter UR periods in activity (Refinetti, 1996) and feeding (Oklejewicz et al., 2001) than did wild-type hamsters.

In neither voles (Gerkema et al., 1993) nor Syrian hamsters (Redlin and Mrosovsky, 1999) were ultrashort light dark cycles that approximate the endogenous periods of URs able to entrain these rhythms. The light-dark cycle does, however, drive other aspects of the UR waveform, notably period (τ') and amplitude (e.g., Gattermann, 1985). The amplitude of the body temperature (T_b) UR of male hamsters was substantially elevated during the D compared with the L phase and also during subjective night in hamsters housed in continuous illumination (LL) or darkness (DD; Refinetti, 1994).

Distinct URs in hormone secretion have been described for glucocorticoids, gonadal steroids, growth hormone (GH), gut peptides, and gonadotropins, among others (Veldhuis, 2008). GH is secreted at intervals of ~1.2 h in male and female Syrian hamsters, with greater UR amplitude in the dark phase (Laartz et al., 1994; Boreratal., 1982). Disproportionately few studies of URs have been conducted on females, a sex bias widespread in biomedical research (Beery and Zucker, 2011).

The influence of hormones on circadian and circannual rhythms is well documented (reviewed in Butler et al., 2009; Zucker, 2001), with ovarian steroids influencing many aspects of circadian organization in Syrian hamsters (Morin et al., 1977; Takahashi and Menaker, 1980; Morin, 1980). Because of the pervasive influence of hormones on several biological rhythms and the functional significance of ultradian organization for control of adaptive behavior (e.g., ovulation, Knobil, 1999), we assessed the influences of the estrous cycle, pregnancy, and lactation, events accompanied by well-defined changes in the endocrine milieu, on Syrian hamster URs.

The female Syrian hamster has a precise 96 h estrous cycle easily monitored from the visible vaginal discharges every fourth day. Because the amplitude of URs is more pronounced in hamsters maintained in a short than long photoperiod (Heldmaier et al., 1989), we monitored females in short day lengths prior to the cessation of estrous cycles and encompassing the window during which females can still be impregnated (Beery et al., 2008).

MATERIALS AND METHODS

Animals and Housing

Syrian hamsters descended from stock (*Mesocricetus auratus*, HsdHan:AURA) obtained from Harlan Sprague-Dawley (Haslett, MI), were housed in polypropylene cages (48 × 25 × 15 cm) on Tek-Fresh bedding (Harlan Teklad, Madison, WI). Hamsters were maintained in long day photoperiods (14 h light per day; LD) and short day photoperiods (10 h light per day; SD) as specified below. Dark onset remained fixed at 1600 h PST in both photoperiods. Food (Prolab RMH 3000, Lab Diet, Richmond, IN) and tap water were available ad libitum.

Ambient temperature was $22 \pm ^\circ\text{C}$. All treatments were approved by the Animal Care and Use Committee of the University of California, Berkeley.

Activity Measurements

Home cage locomotor activity was monitored continuously using passive infrared (IR) motion detectors (Coral Plus, Visonic, Bloomfield, CT) positioned ~30 cm above the cage floor. Motion detectors registered activity when IR beams were crossed. Activity triggered closure of an electronic relay, with cumulative activity counts binned at 10-min intervals and recorded by a computer running Minimitter software (Minimitter, Bend, OR).

Many studies of URs measure foraging or feeding behavior (Daan and Slopeema, 1978; Gerkema et al., 1990, 1993; van der Veen et al., 2006). We measured URs and CRs of spontaneous general locomotor activity—a non-food specific behavior—in an effort to assess estrous and pregnancy effects on rhythmic behavior. Circadian locomotor activity correlates highly with daily rhythms of sleep-wakefulness, body temperature, and drinking behavior (Rusak and Zucker, 1979; Kriegsfeld et al., 2002); in the ultradian domain, locomotor activity correlates positively with feeding rhythms (Gerkema et al., 1993). Locomotor activity studies therefore permit insight into qualitative and quantitative aspects of underlying circadian and ultradian timing systems. Hereafter, when referring to “URs” and “CRs” we are referencing locomotor behavior rhythms

Activity Analyses

Ultradian rhythms—Data were parsed into light-phase activity (light phase: 0600–1600 h; 60 data points per 24 h) and dark-phase activity (dark phase: 1600–06:00 h; 84 data points per 24 h). For each hamster, successive days of photophase-specific activity data were concatenated into a single file from multiple (6–10) consecutive days or nights and subjected to Lomb-Scargle periodogram and cosinor periodogram analyses.

Circadian rhythms—Unparsed files (144 data points per 24 h) 6–10 days in length were subjected to Lomb-Scargle and cosinor periodogram analyses. In all cases, an experimental “day” was defined as beginning at the time of light onset.

Experiment 1: Effects of the Estrous Cycle on Ultradian and Circadian Rhythms

Hamsters exhibiting repeated 4-day estrous cycles ($n = 15$) were transferred to SD at 10 weeks of age. The majority of females maintain estrous cycles and mating behavior for 5–8 weeks after transfer to SD (Beery et al., 2007). Only data from hamsters that continued to exhibit consistent 4-day estrous cycles through 6 weeks in SD were analyzed ($n = 14$).

Consistency and stage of the estrous cycle were determined via daily monitoring shortly after light onset for the presence of a postestrous discharge (PED) (Orsini, 1961) evident on the morning of the day of ovulation, designated day 1 of the vaginal cycle (estrus) (Wise, 1974; McCormack et al., 1998). Days 2, 3, and 4 constitute metestrus, diestrus, and proestrus, respectively, with female sexual receptivity beginning on the evening of proestrus (night 4).

Activity data from cycling females were collected for 9–10 complete and successive 4-day estrous cycles. Data were binned separately for each day of the cycle and subject to UR and CR analyses, as described above.

Experiment 2: Effects of Gestation and Lactation on Ultradian and Circadian Rhythms

Hamsters exhibiting invariant 4-day estrous cycles were transferred to SD at 10 or 13 weeks of age. At 16 weeks of age (after 3–6 weeks in SD), females were mated. On the day before

expected PED (day 4), a LD-housed male was placed in the female's home cage 1.5 h before onset of darkness. For pregnant females ($n = 13$), the day of conception was known on the basis of direct observation. Females that did not produce a litter were classified as nonpregnant ($n = 10$). Litters were weaned at 21 days of age. Recording of locomotor activity of dams and nonpregnant yoked females was terminated 28 days after weaning of young.

Activity data from 8 intervals spanning the female reproductive cycle were subject to UR and CR analyses: prior to mating (PM; 6 days, ending the day before mating), gestational days 1–6 (G1–6), gestational days 6–11 (G6–11), gestational days 11–16 (G11–16), lactational days 1–7 (L1–7), lactational days 8–14 (L8–14), postweaning days 1–7 (PW1–7), and postweaning days 22–28 (PW22–28). Data from postnatal days 15–21 were not analyzed because pups generate locomotor activity during this interval (Guerra and Nunes, 2001), contaminating dam activity recordings. Data from nonpregnant females were collected during yoked time intervals.

Statistical Analyses

Lomb-Scargle periodogram (LSP) analyses were performed to identify the statistical presence or absence of URs and CRs and the number of significant peaks in the UR spectrum (range: 0.1–7.9 h). The level of statistical significance (α) was set to 0.01 for analysis of unparsed (144 data points per day) files and to 0.005 for the analysis of shorter, parsed (60 or 84 data points per day) files to protect against Type I error associated with smaller numbers of data points. Cosinor analyses were used to determine several quantitative measures of URs (range: 0.1–7.9 h) and CRs (range: 22–26 h): robustness (or “prominence,” the percentage of the variance accounted for by the best-fit cosine model, which corresponds to the coefficient of determination R^2 in regression analyses; Refinetti et al., 2007), mesor (rhythm-adjusted mean value around which the waveform oscillates), amplitude (the difference between the peak or trough value and the mesor), and acrophase (the average time [relative to the onset or offset of light] at which the CR waveform exhibits its singular peak, or the UR waveform exhibits its first peak). The level of statistical significance was set to 0.05 and corrected for multiple comparisons. Because α was lower in LSP analyses relative to cosinor analyses, waveforms that failed to meet statistical significance in LSP analyses may still yield significant estimates of waveform parameters in cosinor analyses. Further details of these statistical analyses are in the supplementary online material in the Materials and Methods section.

ANOVAs and pairwise comparisons were performed on a PC with Statview 5.0 (SAS Institute, Cary, NC); LSP and cosinor analyses were performed using software written by R. Refinetti and available at <http://www.circadian.org/software.html> (Refinetti et al., 2007). In both experiments, the proportion of hamsters displaying URs and CRs was evaluated using chi-square tests. In Experiment 1, changes in URs and CRs were assessed across days of the estrous cycle with repeated-measures ANOVAs; pairwise comparisons were performed using paired t tests. In Experiment 2, hamsters in short days for 3 and 6 weeks were statistically indistinguishable in all quantitative aspects of CRs and URs ($p > 0.05$); their data were combined for subsequent analyses. URs and CRs were evaluated for each analysis interval using ANOVA, with parity (pregnant, nonpregnant) as the independent variable. Pairwise comparisons were performed with Fisher PLSD tests and t tests, where appropriate. Differences were considered significant if $p < 0.05$.

RESULTS

Experiment 1: Estrous Cycle Effects on Ultradian and Circadian Rhythms

Ultradian rhythms—On estrous cycle nights 1 and 2 (estrus and metestrus), dark phase URs were present in more than 90% of individuals, but on nights 3 and 4 (diestrus and proestrus), they were detected in only 36% and 21% of hamsters, respectively ($\chi^2 > 9.95$, $p < 0.005$, all comparisons; Fig. 1A). In contrast, the incidence of URs in the light phase was low (36% of hamsters) and did not vary over the estrous cycle ($\chi^2 < 1.7$, $p > 0.20$, all comparisons; Fig. 1A).

UR period (τ') was significantly longer in the D phase relative to the L phase ($F_{1,73} = 17.9$, $p < 0.001$) but was unaffected by stage of the estrous cycle ($F_{3,73} = 0.20$, $p > 0.80$; Fig. 1B). UR acrophase varied systematically ($F_{3,39} = 2.81$, $p < 0.05$), occurring significantly earlier on night 4 than on nights 1 and 2 ($p < 0.05$, all comparisons; Fig. 1C). UR waveforms ($F_{3,39} = 15.4$, $p < 0.001$) were more complex on nights 1 and 2, relative to nights 3 and 4 ($p < 0.001$, all comparisons; Fig. 1D). UR robustness ($F_{3,39} = 17.0$, $p < 0.001$; Fig. 1E), mesor ($F_{3,39} = 49.5$, $p < 0.001$; Fig. 1F), and amplitude ($F_{3,39} = 16.9$, $p < 0.001$; Fig. 1G) all changed over the estrous cycle, exhibiting decreased robustness and amplitude and greater mesor values on nights 3 and 4 ($p < 0.005$, all comparisons). None of the UR parameters measured during the light-phase varied significantly over the estrous cycle ($F_{3,39} < 1.9$, $p > 0.15$, all comparisons; Fig. 1).

Circadian phase affected all UR parameters (Fig. 1). URs were less evident in the light than the dark phase on days 1 and 2 of the estrous cycle ($\chi^2 > 9.95$, $p < 0.005$, all comparisons); significant main effects of circadian phase were evident on UR complexity ($F_{1,104} = 17.1$, $p < 0.001$), τ' ($F_{1,104} = 17.9$, $p < 0.001$), robustness ($F_{1,104} = 5.34$, $p < 0.05$), mesor ($F_{1,104} = 381$, $p < 0.001$), amplitude ($F_{1,104} = 105$, $p < 0.001$), and acrophase ($F_{1,104} = 8.00$, $p < 0.001$).

Circadian rhythms—CRs varied markedly as a function of cycle stage (Figs. 2 and 3). Representative records (Fig. 2A, 2B) illustrate that overall activity levels changed ($F_{3,39} = 65.6$, $p < 0.001$; Fig. 3A) and were lowest on days 1 and 2, at an intermediate level on day 3, and highest on day 4 ($p < 0.001$, all comparisons; Fig. 3A). This primarily reflected changes in the dark phase ($F_{3,39} = 73.3$, $p < 0.001$); activity during the light phase did not vary over the estrous cycle ($F_{3,39} = 0.9$, $p > 0.40$; Fig. 3A). CR robustness ($F_{3,39} = 157$, $p < 0.001$; Fig. 3B), mesor ($F_{3,39} = 66.8$, $p < 0.001$; Fig. 3C), and amplitude ($F_{3,39} = 63.6$, $p < 0.001$; Fig. 3D) all varied over the estrous cycle, with higher values evident on days 3 and 4 ($p < 0.05$, all comparisons) as did timing of the acrophase ($F_{3,39} = 5.4$, $p < 0.005$; Fig. 3E), with a significant delay on day 4 relative to all other cycle days. Visual inspection of the actograms revealed substantially more consolidation of nocturnal activity on day 4 (proestrus) compared with other days (Fig. 2A).

Experiment 2: Reproductive Cycle Effects on Ultradian and Circadian Rhythms

Ultradian rhythms—Locomotor activity of dams and yoked nulliparous females was monitored continuously, beginning prior to mating. All aspects of light and dark phase URs were indistinguishable between pregnant and nonpregnant dams prior to mating ($p > 0.10$, all comparisons; Fig. 4A–E; Suppl. Fig. S1A, B).

Prior to mating, D phase URs were present in ~40% of females (Fig. 4A). The proportion of individuals with detectable D phase URs increased significantly during early ($\chi^2 = 7.1$, $p < 0.01$) and late ($\chi^2 = 5.3$, $p < 0.05$) gestation and decreased below pre-mating levels after

parturition (L1–7: $\chi^2 = 6.3$, $p < 0.05$; Fig. 4A). Gestation, lactation, and weaning were without effect on the incidence of light phase URs ($\chi^2 < 1.9$, $p > 0.10$, all comparisons).

Parity significantly altered UR complexity ($F_{1,336} = 11.4$, $p < 0.001$; Fig. 4C), mesor ($F_{1,336} = 10.3$, $p < 0.005$; Suppl. Fig. S1A), and amplitude ($F_{1,336} = 16.8$, $p < 0.001$; Fig. 4E) and the pattern of change in UR robustness over successive stages of the reproductive cycle (parity \times time: $F_{7,336} = 3.59$, $p < 0.001$). There was a main effect of pregnancy on τ' of dark phase ($F_{1,140} = 4.05$, $p < 0.05$; Fig. 4B) but not light phase URs ($F_{1,82} = 1.98$, $p > 0.15$). No omnibus effect of parity was evident on UR acrophase ($F_{1,222} = 0.02$, $p > 0.90$; Suppl. Fig. S2B).

UR τ' during the dark phase tended to be shorter late in gestation (G11–16: $p = 0.058$; Fig. 4B) and was shorter among the few dams exhibiting D phase URs in late lactation (L8–14: $p < 0.05$). No significant effects of parity were evident on L phase τ' (all comparisons).

Dark phase UR waveforms became more complex during gestation ($p < 0.05$, all comparisons; Fig. 4C) and less complex during early lactation ($p < 0.005$). UR complexity increased during the early postweaning interval (PW1–7: $p < 0.001$; Fig. 4C). Complexity of L phase URs did not vary over the reproductive cycle ($p > 0.30$, all comparisons).

Dark phase UR robustness was singularly enhanced throughout gestation ($p < 0.05$, all comparisons; Fig. 4D) but during lactation robustness was significantly decreased ($p < 0.05$). Upon weaning of young, UR robustness of dams was indistinguishable from that of controls ($p > 0.40$, all comparisons).

UR amplitude was significantly increased during mid- and late gestation ($p < 0.05$, both comparisons) but only in the D phase (Fig. 4E). During lactation, UR amplitude decreased in both the D phase (L8–14, $p < 0.005$) and L phase (L1–7 and L8–14, $p < 0.01$, both comparisons; Fig. 4E illustrated for D-phase data only). In both photophases, amplitude suppression of URs was resolved within the first week after weaning.

UR mesor values were comparable in pregnant and nonpregnant females throughout pregnancy ($p > 0.30$, all comparisons; Suppl. Fig. S1A); during lactation, however, UR mesor values increased sharply in both the D (L8–14, $p < 0.001$) and L phases (L1–7 and L8–14, $p < 0.01$, both comparisons; see Suppl. Fig. S1A for D phase data).

Circadian rhythms—All hamsters exhibited CRs prior to mating (PM) and throughout gestation (G1–6, G6–11, and G11–16 intervals; Figs. 5, 6) but CRs were absent in a significant proportion of dams during the first (L1–7; $\chi^2 = 7.7$, $p < 0.01$) and second (L8–14; $\chi^2 = 3.7$, $p = 0.05$) weeks of lactation (Fig. 6A). CRs reappeared in all previously arrhythmic parous females within the first week after weaning (PW1–7).

CR robustness declined during gestation ($F_{1,168} = 48.0$, $p < 0.001$; Fig. 6B), evident in the first 6 days of pregnancy (G1–6: $p < 0.005$), and persisted through mid-gestation (G6–11: $p < 0.01$) and late gestation (G11–16: $p < 0.001$); CR robustness was further diminished at the onset of lactation ($p < 0.001$, both comparisons). CR amplitude ($F_{1,162} = 106.8$, $p < 0.001$; Fig. 6C) was markedly lower in parous females during gestation ($p < 0.05$, all comparisons) and lactation ($p < 0.001$, all comparisons) but rebounded after young were weaned. Parity affected CR mesor ($F_{1,162} = 18.7$, $p < 0.001$; Suppl. Fig. S2A), but this main effect reflected increased activity during the mid-lactation period (L8–14), presumably representing the emergence of pup activity at 10–12 days of age (Guerra and Nunes, 2001). CR acrophase was progressively more delayed over time in dams and control females alike ($F_{7,162} = 5.79$, $p < 0.001$) and was unaffected by parity ($F_{1,162} = 0.60$, $p > 0.70$; Suppl. Fig. S2B). Changes

in all quantitative aspects of CRs evident in parous females during gestation and lactation were reversed upon weaning (Fig 6A–C; Suppl. Fig. S2A–B).

DISCUSSION

Marked changes in period, robustness, complexity, amplitude, and phase of hamster ultradian and circadian behavior were associated with successive stages of the estrous cycle, gestation, lactation, and weaning. This is the first documentation of reproductive modulation of URs in home cage activity; passive monitoring of activity is a more ecologically valid measure of general activity than is wheel running. In the present context, the limitations of wheel running would be prohibitive for understanding how URs change. When female Syrian hamsters are provided access to a running wheel, their activity declines beginning 1 day after mating, with progressive decreases from day 2 to 16 of pregnancy, culminating in its virtual absence (Richards, 1966). By contrast, high levels of home cage activity were sustained throughout gestation when monitored with motion detectors (Fig. 5B) and by Richards (1966) via direct observation of dams. Syrian hamsters do not engage in wheel running behavior during the light phase (Zucker and Stephan, 1973; Aschoff et al., 1973) but generate substantial light-phase locomotor activity when monitored with a spring-suspended tilt cage (Aschoff et al., 1973). Thus, measurement of light-phase URs via running wheels is not the method of choice.

Ultradian Rhythms

Estrous cycle—Categorical and quantitative aspects of locomotor activity URs varied with stage of the estrous cycle during the dark but not the light phase of the 24-h light-dark cycle. Estrous cycle influences on locomotor activity URs were absent in intact female LEW/Ztm rats monitored with motion detectors (Wollnik and Döhler, 1986); an ultradian pattern of wheel-running on metestrus and diestrus but not on estrus subsequently was reported (Wollnik and Turek, 1988), which differs from the highest incidence of URs on estrus and metestrus in the present study. In both rats and hamsters, the dominant behavioral UR recurs at intervals of 4–5 h, suggesting that changing patterns of hormone secretion over the estrous cycle influence the substrate that generates URs. Although SCN electrical activity oscillates with significant ultradian periods (Meijer et al., 1997), behavioral URs persist in rats, hamsters, and voles with SCN lesions that eliminate circadian rhythms (Eastman et al., 1984; Refinetti, 1994; Gerkema et al., 1990).

Pregnancy and lactation—The proportion of hamsters with significant dark phase URs increased during gestation and decreased during lactation. Parity significantly altered dark phase UR complexity, mesor, and amplitude; the period of the UR tended to be shorter late in gestation and in the few dams exhibiting URs in late lactation. Disruption of the dams' activity pattern during lactation undoubtedly reflects the extensive demands of nursing, during which dam movement is curtailed (Guerra and Nunes, 2001) and exhibits significantly less UR robustness. UR amplitude was restored in the first week after weaning, which could reflect postweaning hormonal changes or the elimination of pup-dam interactions. The extent to which hormonal status during lactation contributes to this striking modification of ultradian organization remains to be investigated.

Disentanglement of the respective roles of ovarian estradiol (E) and progesterone (P) in mediating UR fluctuations remains a significant challenge. In nonpregnant, cycling hamsters, P rises abruptly on day 4 (Saidapur and Greenwald, 1978), and E rises on days 3 and 4 (Baranczuk and Greenwald, 1974) of the estrous cycle, but UR prominence is lowest on these days. This lack of correspondence between times of elevated E and P and prominent URs over the estrous cycle is difficult to reconcile with a simple model in which

ovarian steroids facilitate URs. A further consideration is the marked increase in activity consolidated into a single bout during night 4 (and to a lesser extent, night 3) of the estrous cycle, which may mask URs. Visual inspection of the wheel-running records of female Sherman rats confirms the absence of URs during estrus (Gerall et al., 1973).

Circadian Rhythms

Estrous cycle—Estrogens shorten the period of the free-running wheel-running rhythm of ovariectomized female (Morin et al., 1977; Takahashi and Menaker, 1980) but not male hamsters (Zucker et al., 1980) and phase advance activity onset on 2 of the 4 days of the estrous cycle (Morin et al., 1977). The present study extends these observations to home cage activity and reveals that changes in locomotor activity over the estrous cycle are attributable to activity occurring exclusively during the dark phase of the photocycle. CR robustness, mesor, and amplitude all increase on cycle days 3 and 4, with CR acrophase delayed on day 4 relative to other cycle days, presumably reflecting changes in the pattern of estradiol secretion.

In male hamsters, short photoperiods decrease locomotor activity (Ellis and Turek, 1983; Morin and Cummings, 1981) and increase the amplitude of the circadian phase-response curve to light (Pittendrigh et al., 1984; Evans et al., 2004). If comparable sequelae are evident in female hamsters in short days, then the magnitude of estrus-induced changes in locomotor activity levels in long days may be substantially greater than those reported here.

Pregnancy and lactation—CRs in locomotor activity were sustained throughout gestation but were absent in a significant proportion of dams during lactation. The decline in CR robustness evident in the first 6 days of pregnancy persisted throughout gestation and was further diminished at the onset of lactation only to be rapidly reversed upon weaning. Additional studies are required to assess the respective roles of ovarian and pituitary hormone secretion, and fetal/ pup signals, on maternal CRs during these life stages. Our study did not include evaluation of τ , as hamsters were entrained to a light-dark cycle; however, progressive phase-delays in acrophase in dams between the first and last day of gestation may indicate a τ -lengthening effect of pregnancy.

Summary

CRs are diminished and robust behavioral URs emerge coincident with estrus, and a progressive diminution of CRs and emergence of URs occur over gestation. Mechanistically, changes in gonadal steroids may be causally related to changes in rhythmic behavior, an issue that can be addressed by hormone withdrawal and replacement regimens.

Estrous cycle-induced changes in CR robustness and amplitude occur in a pattern inverse to that of URs: URs are most prominent when CRs are least robust and vice versa. A similar association is observed over the course of gestation. Reproduction-related decrements in CRs may be necessary for URs to emerge; alternatively, estrus and pregnancy may independently induce parallel changes in the circadian and ultradian systems. Emergence of URs occurs after attenuation or elimination of CRs accomplished via brain lesions (Eastman et al., 1984; Rusak, 1977; Refinetti, 1994; Gerkema et al., 1990) or by exposure to artificial (Honma and Hiroshige, 1978) or natural (van Oort et al., 2007) constant light. The present data extend this association between CRs and URs to naturally occurring events in the female reproductive cycle.

The adaptive significance of several URs is well established. In female rhesus monkeys, the approximately 60-min UR in GnRH secretion is a *sine qua non* for ovulation (Knobil, 1999). The common vole (*Microtus arvalis*) has prominent locomotor and feeding rhythms with a

period of ~2 h. Synchronization of these URs among members of the population markedly decreases the risk of predation for any given vole (Daan and Slopsema, 1978). The behavior of free-ranging Svalbard reindeer (*Rangifer tarandus*) is circadian arrhythmic during the polar day but exhibits robust ultradian rhythms with periods of approximately 6–7 h (van Oort et al., 2007). This species lacks a functional fibroblast circadian clock and may also lack functional molecular clockworks in the brain (Lu et al., 2010). Weak circadian organization of behavior may be an adaptation that permits around-the-clock feeding in an environment that exhibits dramatic changes in photoperiod.

At present, the adaptive significance of estrus- and pregnancy-induced changes in UR prominence remains to be established but may be related to patterns of activity that promote successful mating and care of the young. Fitness benefits may accrue to individuals capable of abandoning rigid circadian-dominated control of behavior in favor of greater behavioral flexibility—characterized by a stronger ultradian structure—during life stages when interactions with mates and offspring are less predictable.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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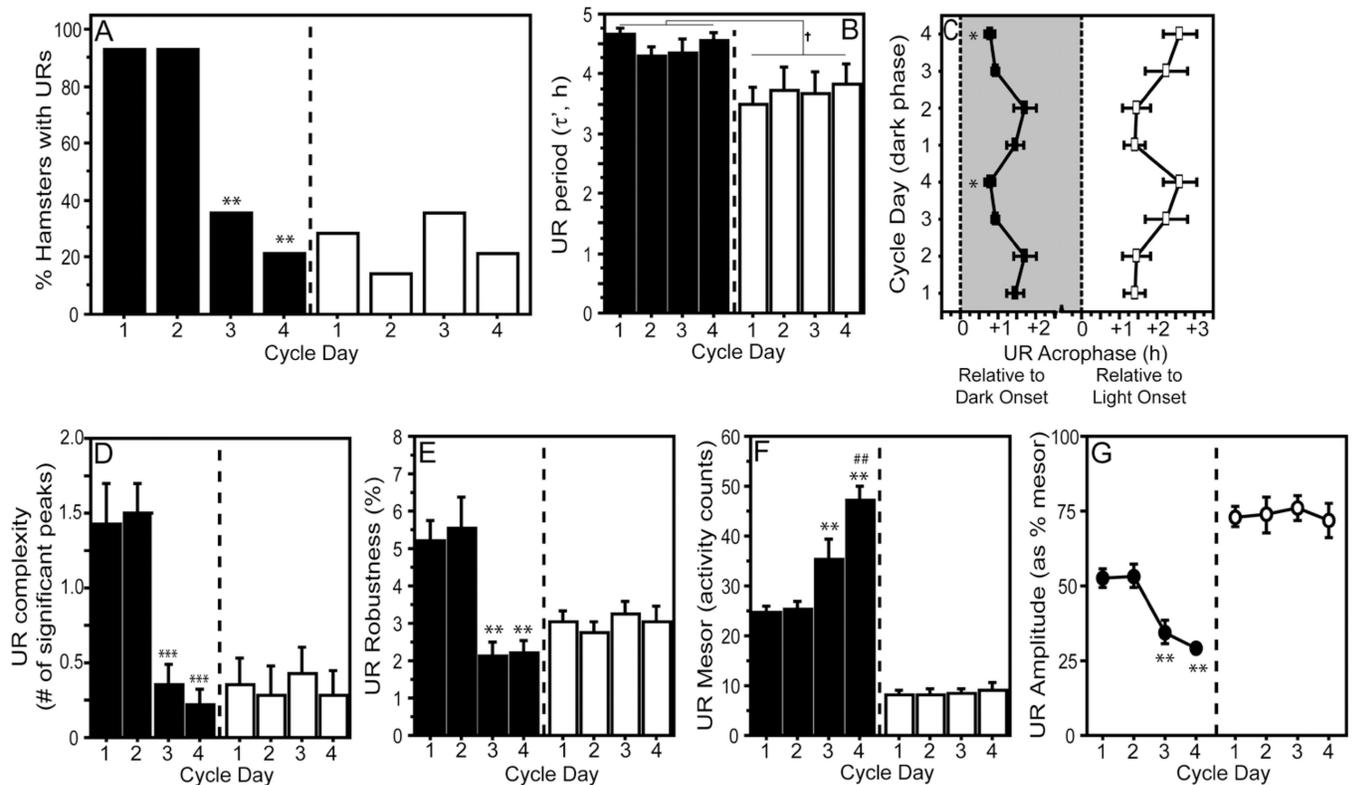


Figure 1.

Dark and light phase ultradian activity across the estrous cycle. (A) Percentage of hamsters exhibiting significant ultradian rhythms. (B) Mean \pm SEM period (τ'), (C) acrophase, (D) complexity (number of significant periods), (E) robustness, (F) mesor, and (G) amplitude of the dark phase and light phase ultradian waveforms. Dark phase data are depicted on the left (in black), and light phase data on the right (in white). * $p < 0.05$, ** $p < 0.005$, *** $p < 0.001$ vs. nights 1 and 2; ## $p < 0.005$ vs. night 3. † $p < 0.001$ vs. dark phase.

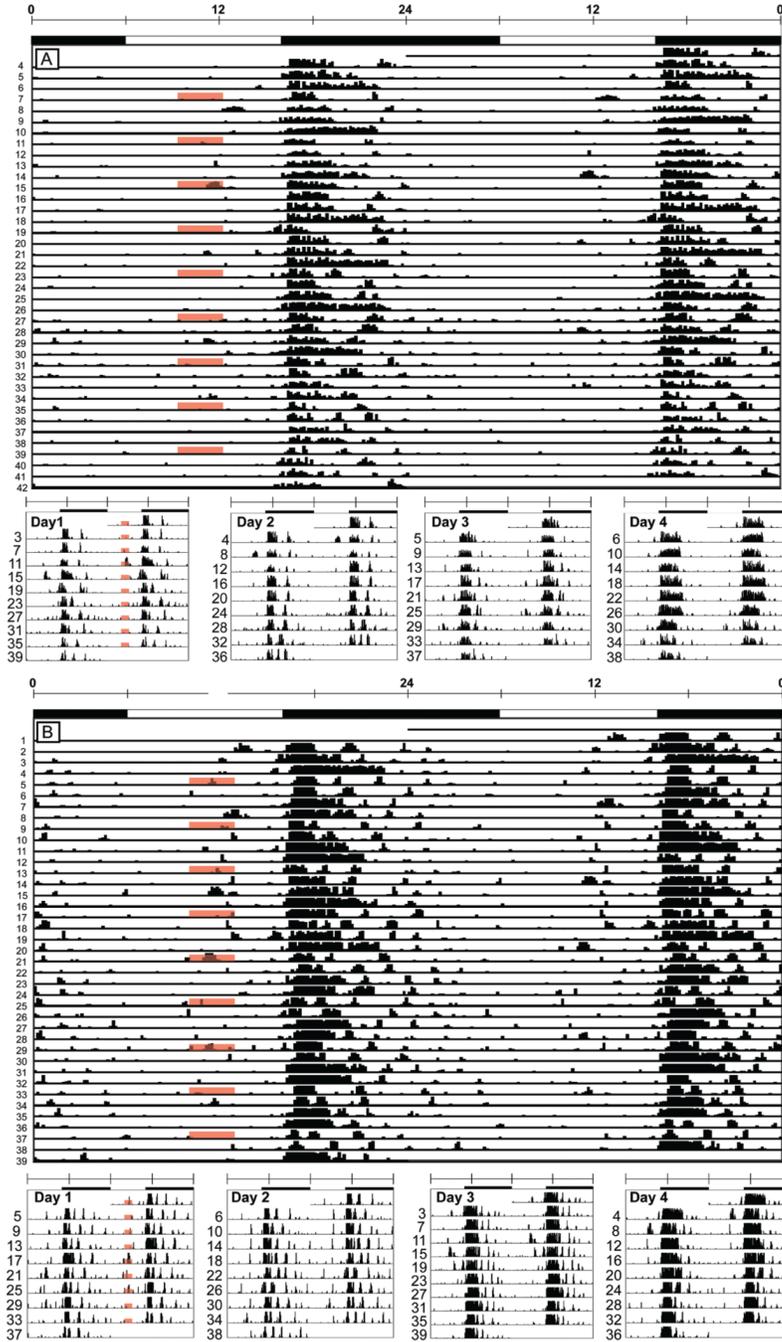


Figure 2. Circadian locomotor activity during the estrous cycle. Representative double-plotted activity records of 2 hamsters. Clock time is indicated on the horizontal axis at the top of each actogram, along with light (white) and dark (black) phases of the 10-h light, 14-h dark photoperiod. Below each continuous actogram are 4 records in which activity is binned and replotted by day of the estrous cycle for 10 consecutive cycles, for an individual hamster. Estrous cycle days are indicated at the top of each actogram (1 = day of ovulation; 4 = day of proestrus). The morning of postestrus vaginal discharge is indicated by a shaded area on the record.

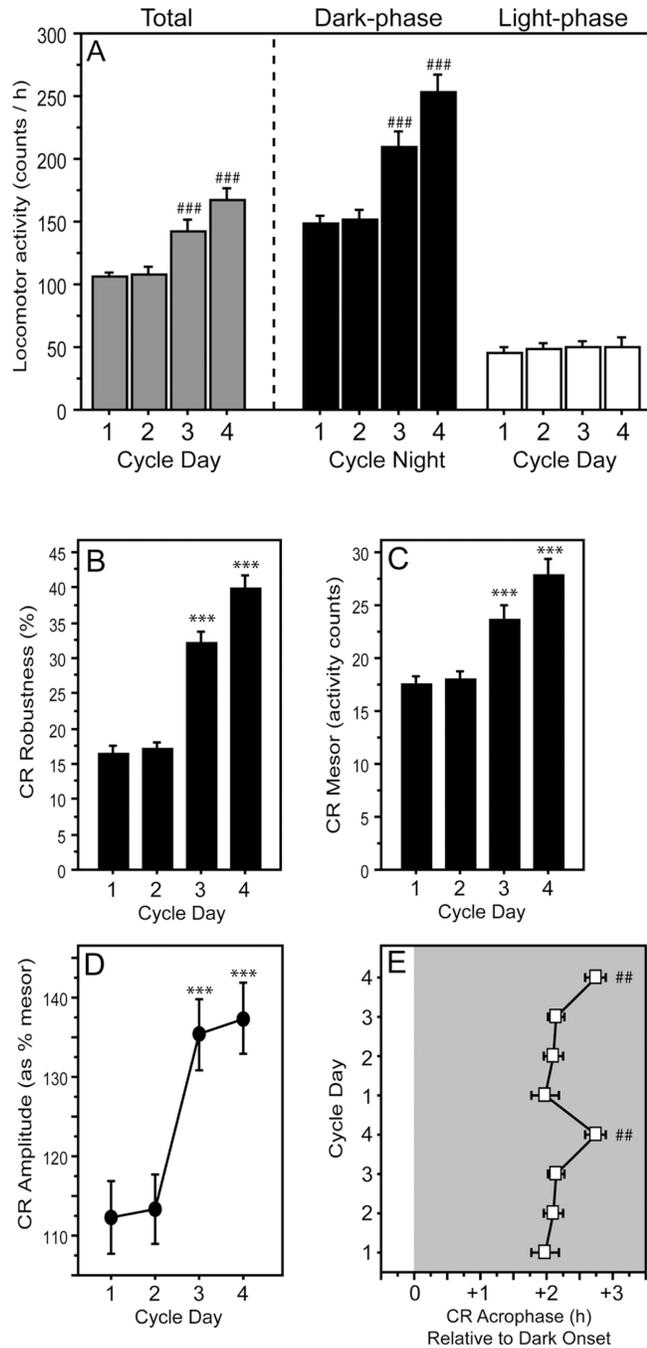


Figure 3.

Components of circadian activity over the estrous cycle. (A) Mean \pm SEM activity counts per hour over successive days of the cycle. Data to the left and right of the dashed line depict activity over the 24-h day and activity restricted to the dark and light phases, respectively. (B) Mean \pm SEM robustness, (C) mesor, (D) amplitude (as percentage of mesor value), and (E) acrophase of the circadian waveform. $##p < 0.01$ and $###p < 0.001$ vs. all other days; $***p < 0.001$ vs. days 1 and 2.

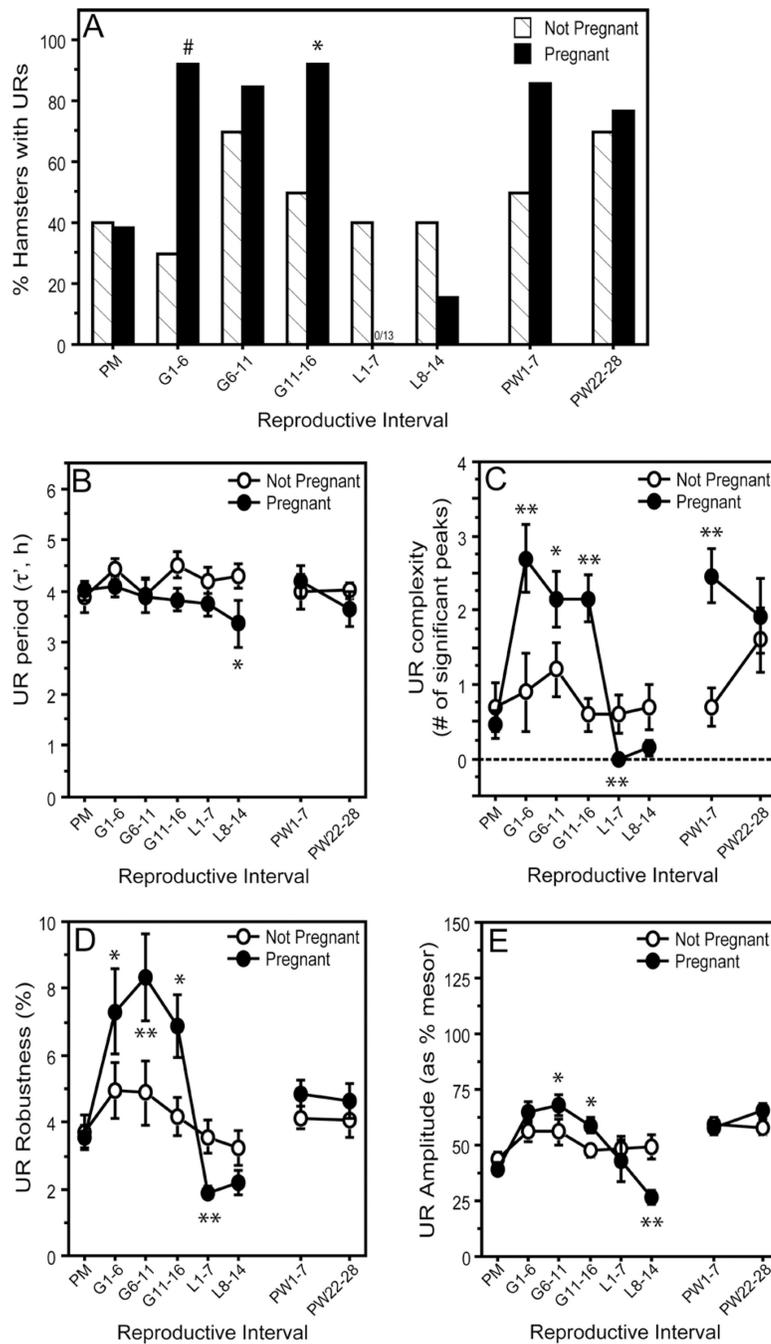


Figure 4. Dark phase ultradian rhythms over the reproductive cycle. (A) Percentage of hamsters exhibiting significant dark phase URs. (B) Mean \pm SEM period (τ'), (C) complexity, (D) robustness, and (E) amplitude of the ultradian waveform ^{*} $p < 0.05$, [#] $p < 0.01$, ^{**} $p < 0.005$ vs. “Not Pregnant” group.

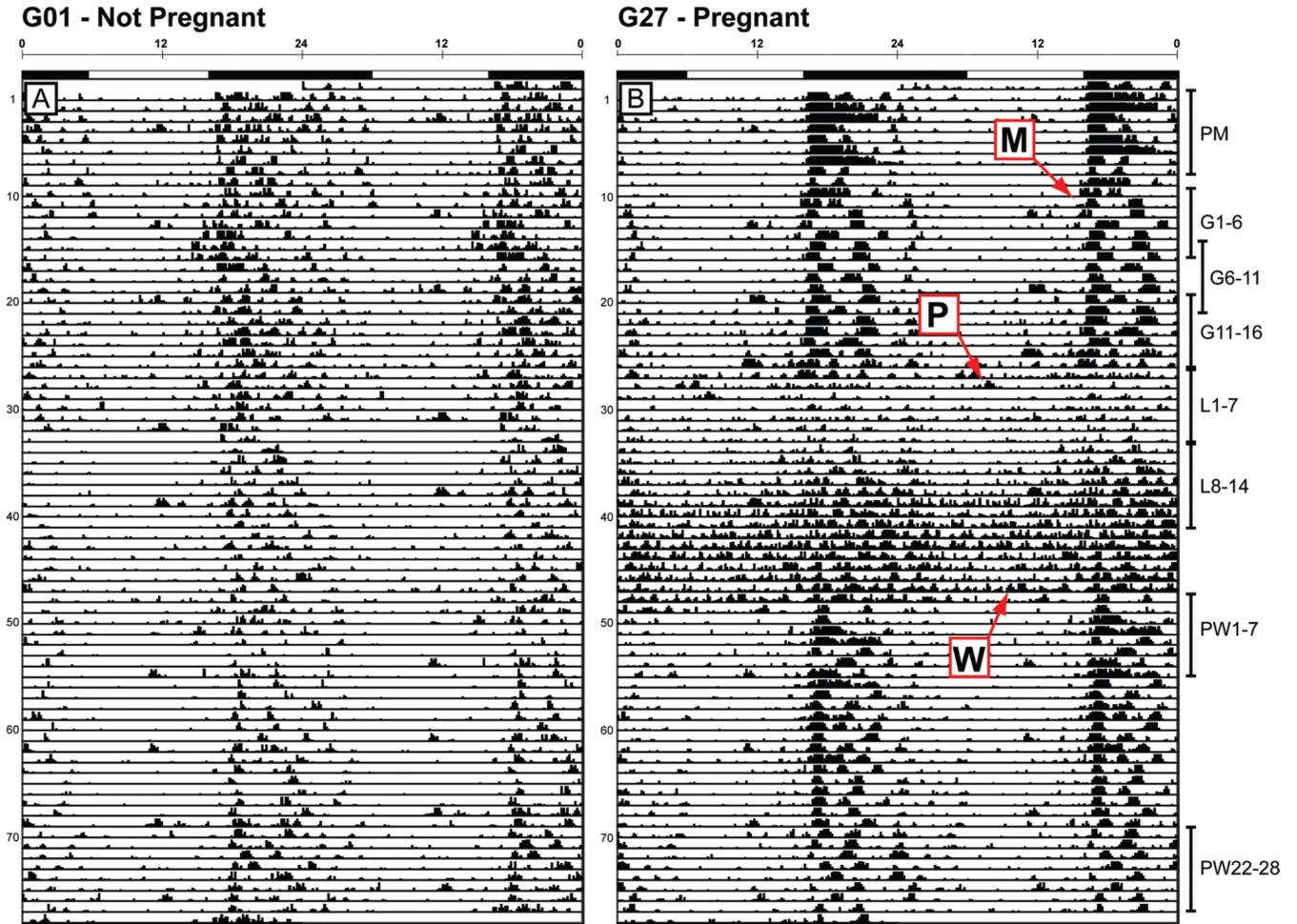


Figure 5. Circadian locomotor activity over successive stages of the reproductive cycle. Representative double-plotted activity records of a nonpregnant (A) and a pregnant (B) hamster. Clock time is indicated on the horizontal axis at the top of each actogram, along with light (white) and dark (black) phases of the 10:14 photoperiod. In Panel B, the letters *M*, *P*, and *W* indicate the time of mating, parturition, and weaning, respectively. Intervals subjected to data analysis are indicated to the right of Panel B: PM = premating, G1-6 = gestational days 1–6, G6-11 = gestational days 6–11, G11-16 = gestational days 11–16, L1-7 = lactation days 1–7, L8-14 = lactation days 8–14, PW1-7 = postweaning days 1–7, PW22-28 = postweaning days 22–28.

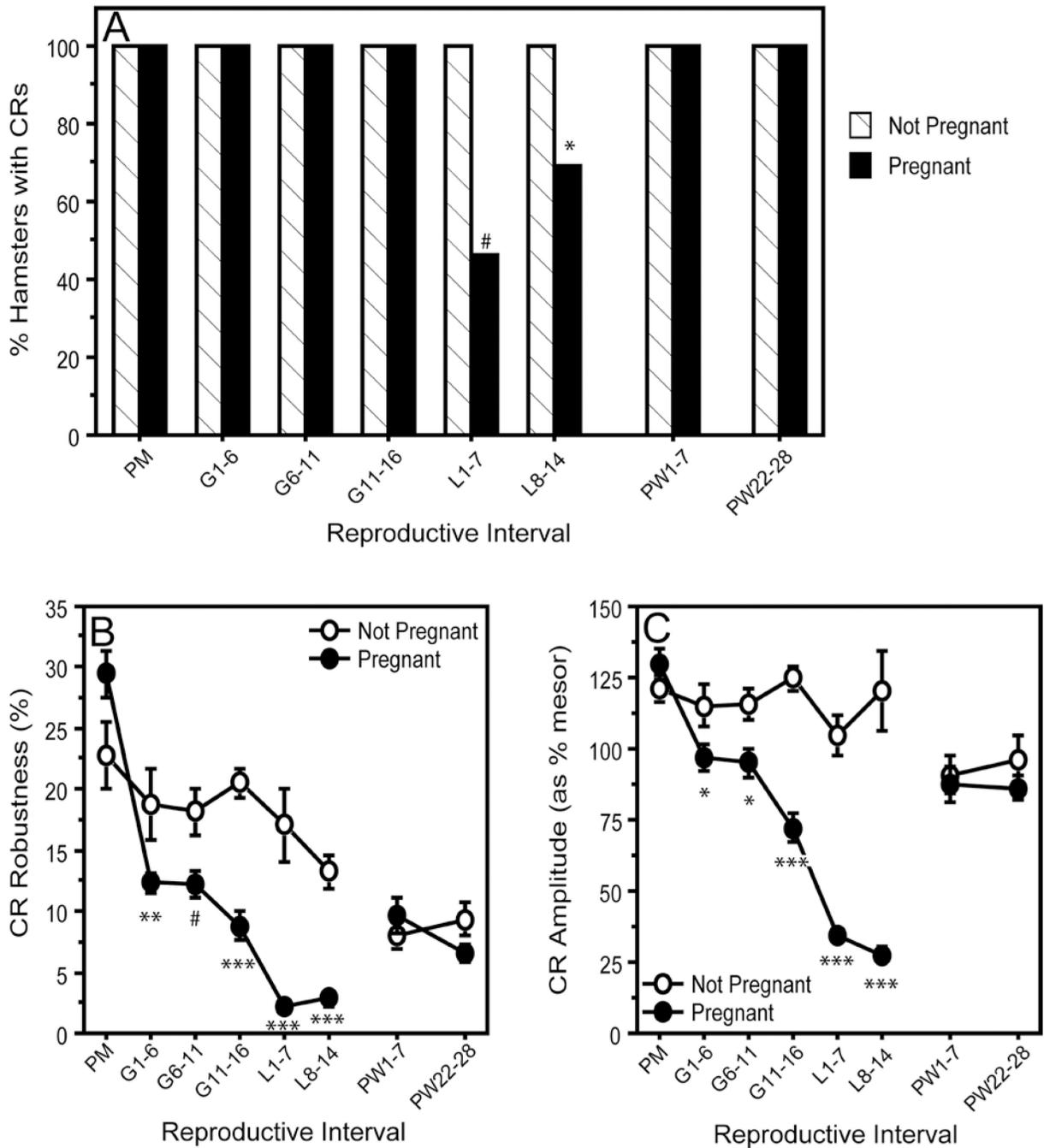


Figure 6. Components of circadian activity rhythms over the reproductive cycle. (A) Percentage of hamsters exhibiting significant circadian rhythms. (B) Mean \pm SEM robustness, and (C) amplitude of the circadian waveform. * $p < 0.05$, # $p < 0.01$, ** $p < 0.005$, *** $p < 0.001$ vs. "Not Pregnant" group.