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Pregnancy-induced changes in ultradian rhythms persist in circadian arrhythmic Siberian hamsters

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Abstract

The impact of pregnancy and lactation on ultradian rhythms (URs) and circadian rhythms (CRs) of locomotor activity was assessed in circadian rhythmic and arrhythmic Siberian hamsters maintained in a long-day photoperiod (16 h light/day). Progressive decrements in CR robustness and amplitude over the course of gestation were accompanied by enhanced URs. Dark-phase UR period and amplitude increased during early gestation and complexity and robustness increased during late gestation. The persistence of pregnancy-associated enhancements of URs in circadian arrhythmic (ARR) hamsters suggests that reproductive modulation of the UR waveform is not dependent on coherent circadian organization. The increased incidence of dark-phase URs appeared more rapidly in ARR dams than entrained (ENTR) dams. Throughout gestation, the percentage of dams with dark-phase URs was significantly greater in the ARR group. Gestational increases in UR complexity and robustness emerged earlier and were greater in ARR than ENTR dams. The attenuation of CRs during lactation is correlated with increased expression of URs. Relaxation of circadian control of the dam's behavior may increase fitness by permitting more efficient interactions with circadian arrhythmic pups.

Keywords

Biological rhythms; Suprachiasmatic nucleus; Arrhythmia; Reproduction; Lactation; Maternal behavior

Introduction

Ultradian rhythms (URs) impose temporal order on a sub-circadian timescale (typically with periods [τ'] < 8 h) (Shannahoff-Khalsa et al., 2001; Veldhuis, 2008; Walker et al., 2012; Yates and Yates, 2008) that affect diverse aspects of physiology and behavior, including

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hormone secretion (Choe et al., 2013; Knobil, 1999; Lloyd et al., 2008; Veldhuis, 2008), gene expression (Baggs and Hogenesch, 2010), sleep patterns (Mueller et al., 2012), food intake (Warner et al., 2010), body temperature (Heldmaier et al., 1989), and foraging behavior (Blessing et al., 2012).

Unlike circadian, circannual, lunar, and tidal rhythms, URs are not an evolved match to recurring environmental signals (Daan and Aschoff, 1981); attempts to entrain URs with zeitgebers in the ultradian range generally have been unsuccessful (Gerkema et al., 1993; Redlin and Mrosovsky, 1999, but see Luby et al., 2012), although phase setting by sunrise (Madison, 1981) and sunset (Halle, 1995) have been reported for URs of voles in the field. An intrinsic mechanism (see Gerkema et al., 1990) perhaps augmented by homeostatic feedback has been proposed for generation of URs (Halle and Stenseth, 1994). Under some conditions (e.g., lactation), where the demands of the young require maternal care during the dams' usual rest phase, relaxation of circadian control and the emergence of URs may be advantageous (Bloch et al., 2013; Madison, 1978; Nishihara et al., 2002).

Expression of URs does not appear to depend on a functional circadian system. URs persist in rodents with circadian dysfunction induced by suprachiasmatic nucleus (SCN) lesions (Eastman et al., 1984; Gerkema et al., 1990, 1993; Osborne and Refinetti, 1995; Refinetti, 1994; Ruby and Zucker, 1992; Rusak, 1977; Waite et al., 2012), constant light (Deprés-Brummer et al., 1995; Honma and Hiroshige, 1978) and by circadian clock gene mutations (Nakamura et al., 2013).

URs in the 4 h range have been documented in locomotor activity of 11 different mouse strains (Dowse et al., 2010), as well as in sleep-wake rhythms of rats (Stephenson et al., 2012). In studies of those species, as well as of Syrian and Siberian hamsters, investigators employing several different statistical approaches have consistently established UR periods close to 4 h, suggesting that these rhythms are useful adaptations.

Circadian rhythms (CRs) are not, however, without influence on URs. Locomotor activity and drinking behavior URs became more prominent and complex in male Syrian hamsters (*Mesocricetus auratus*) in which CRs were eliminated by destruction of the SCN (Rusak, 1977). Male Siberian hamsters (*Phodopus sungorus*) maintained in short photoperiods exhibited increases in UR period, complexity and amplitude, concurrent with decreases in CR amplitude (Prendergast and Zucker, 2012). Circadian phase likewise altered UR incidence, τ', complexity, and amplitude in female Syrian hamsters (Prendergast et al., 2012a). CRs appear to tonically inhibit the generation and/or expression of URs (Prendergast and Zucker, 2012; Prendergast et al., 2012b). It remains unclear whether residual low-amplitude CRs are a prerequisite for enhancement of URs.

In many organisms, CRs change markedly over the reproductive cycle (Bloch et al., 2013; Morin et al., 1977); limited evidence suggests that URs become more prominent coincident with pregnancy-associated CR decrements (Prendergast et al., 2012a). In Syrian hamsters, CR robustness and amplitude were reduced during gestation, coincident with marked increases in UR robustness and amplitude. Dams for the most part were circadian and ultradian arrhythmic during lactation, with both rhythms in both frequency domains restored

to pre-mating power within a week of weaning offspring (Prendergast et al., 2012a). A transient loss of obligate circadian structure in locomotor activity may be adaptive in dams, whose primary interactions are with arrhythmic offspring that require around-the-clock care (Bloch et al., 2013; Prendergast et al., 2012a). It is not known if gestational modulation of the UR waveform is idiosyncratic to Syrian hamsters, or occurs in most altricial rodents.

Additionally, whether pregnancy-associated changes in URs occur downstream of decrements in CR amplitude also remains unresolved. Siberian hamsters provide a useful model to probe interactions of circadian and ultradian timing. URs in locomotor activity occur at intervals of 3–5 h in Siberian hamsters (Prendergast et al., 2012b). A precisely-timed phase shift of the light–dark cycle (disruptive phase shift; DPS) renders a substantial proportion of hamsters permanently circadian arrhythmic (Ruby et al., 2004). This form of circadian arrhythmia is immune to confounds inherent in rodents rendered arrhythmic with SCN lesions, constant light, or gene mutations. DPS arrhythmic animals retain *c-fos* and *Per1* mRNA responses to light (Barakat et al., 2005), but exhibit low and arrhythmic expression of *Per1*, *Per2*, and *Bmal1* mRNAs in constant darkness (Grone et al., 2011). CRs in locomotor activity, body temperature, sleep, hormone secretion, and cognitive rhythms (Larkin et al., 2004; Ruby et al., 2004; Ruby et al., 2008) are compromised in DPS arrhythmic hamsters, even during maintenance in a light–dark cycle (Prendergast et al., 2012b).

A prior investigation examined the effects of pregnancy and lactation on URs of Syrian hamsters housed in short day lengths. Because day length markedly influences both CRs and URs (Prendergast and Zucker, 2012), the present study sought to extend the investigation of pregnancy-induced changes in CRs and URs to a second hamster species housed in long days.

Material and methods

Animals

Female Siberian hamsters (*P. sungorus*) were from a colony maintained on a long-day, 15 h light:9 h darkness (15 L:9D) photoperiod (lights off at 18:00 h CST) at the University of Chicago. Hamsters were derived from a population maintained by Dr. Katherine Wynne-Edwards (Queen's University, Kingston, ON, Canada). Hamsters were individually housed in polypropylene cages (28 × 17 × 12 cm), with food (Teklad rodent diet 8604, Harlan, Indianapolis, IN) and filtered tap water provided *ad libitum*; cotton nesting material was also available in the cage. Ambient temperature and relative humidity were held constant at 19 ± 2 °C and 53 ± 10%, respectively. All procedures conformed to the USDA Guidelines for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the University of Chicago.

Disruptive phase shift (DPS) procedure

Females were subjected to the DPS manipulation that destabilizes the Siberian hamster circadian pacemaker, rendering a substantial proportion of hamsters permanently circadian arrhythmic ("ARR"; Ruby et al., 2004). Females were housed for at least 4 weeks in 16 L:

8D photoperiod (lights off at 18:00 h CST) prior to undergoing a 2-h light pulse from the 5th to the 7th hours of the 8 h dark phase. The next day, the 16 L:8D photocycle was phase delayed by 3 h by extending the light phase (new lights off at 21:00 h CST). Control hamsters were subjected to the 3 h phase delay but did not receive the 2 h light pulse on the preceding night. The DPS protocol typically induces circadian arrhythmicity in 50% of hamsters, whereas >90% of control hamsters typically retain a normal, circadian entrained ("ENTR") phenotype (Prendergast et al., 2012b; Ruby et al., 2004).

Circadian phenotyping

Circadian phenotypes were identified prior to mating using criteria described in prior reports of DPS-induced arrhythmia (Prendergast et al., 2012b; Ruby et al., 2004, 2008). Hamsters were identified as ENTR or ARR based on the presence/absence of a significant CR in locomotor activity. χ^2 periodogram analyses (ClockLab; Actimetrics, Evanston, IL) were performed on 10 day blocks of activity data, 2–3 months after the phase shift was administered (cf. Ruby et al., 2004). Peaks in the χ^2 periodogram were considered statistically significant if they exceeded the 99.9% confidence interval limit (P < 0.001). Hamsters were considered ARR if there were no significant peaks in the periodogram in the circadian range, with activity distributed throughout the light–dark cycle, and daily activity onsets and offsets could not be identified by visual inspection of the actogram. Hamsters with significant circadian activity in the χ^2 periodogram were considered ENTR (Ruby et al., 2004).

Pregnancy

Females (ENTR: n = 27, ARR: n = 23) were mated by placing a male hamster into each female's home cage for 3 h beginning 1 h before the onset of darkness. The light–dark cycle (16 h light/day; lights off at 21:00 h C.S.T.) was maintained during the mating tests with dim red light illumination to permit animal handling and observation. This procedure was repeated on 5 consecutive evenings to cover the entire Siberian hamster estrous cycle. Because mating trials occurred in a room separate from the one in which locomotor activity was monitored, home-cage locomotor activity was not collected during these 3 h intervals. Beginning 17 days after the first pairing, females were inspected twice daily (morning and evening) for the presence of a litter.

The day of birth, designated P0, was used retrospectively to label 18 days of gestation (G0–17). Pups were weaned on postnatal day 18 (P18). Females subjected to mating tests that failed to produce litters were omitted from all analyses because it was uncertain whether they failed to become pregnant, became pseudopregnant, or were pregnant but resorbed their litters. Females not subjected to the mating procedure (ENTR n = 12, ARR n = 7) served as controls; a simulated parturition date was assigned to control females by yoking their activity records to those of randomly selected parturient dams.

Locomotor activity monitoring

After the DPS treatment, home-cage locomotor activity was assessed with passive infrared motion detectors (Coral Plus, Visonic, Bloomfield, CT) positioned outside the cage (22 cm above the cage floor). URs recorded in this fashion have significantly higher power than

those monitored with activity wheels (van der Veen et al., 2006). Motion detectors registered activity when 3 of 27 zones were crossed. Activity triggered closure of an electronic relay recorded by a computer running ClockLab software. Continuous passive infrared recording (PIR) started at least 9 days prior to mating ("Pre-mating"), continued through mating, gestation, lactation, early post-weaning and 1 month post-weaning. Because Siberian hamster pups exhibit independent locomotor activity beginning around 12 days of age and contribute to activity recorded in the cage, only the dams' activity during the first 10 days of lactation was analyzed. Cumulative activity counts were collected at 6 min intervals.

Light- and dark-phase raw activity data were parsed into light-phase activity (16 h light phase: 160 data points per 24 h) and dark-phase activity (8 h dark phase: 80 data points per 24 h). Successive days of photophase-specific activity data were concatenated into a single file from multiple consecutive days or nights for each hamster and subjected to Lomb–Scargle periodogram (Lomb, 1976) and cosinor periodogram analyses (see *Circadian and ultradian waveform analyses*, below). Unparsed raw activity data files (240 data points per 24 h) were subjected to Lomb–Scargle and cosinor periodogram analyses of CRs.

Activity data were analyzed separately for each of 6 separate intervals across the reproductive cycle: Pre-mating (10 consecutive days within 1 month of mating), early gestation (G0–G8), late gestation (G9–G17), lactation (P0–P9), post-weaning (P19–P28), and 1 month post-weaning (P49–P58). Due to a power failure in the ClockLab computer system, data from the post-weaning and 1 month post-weaning epochs were collected 13 days later than scheduled for a subset of control hamsters (ARR: n = 7, ENTR: n = 8). CR and UR data from the non-mated controls that were tested 13 days later were compared to those from non-mated controls that were tested at the prescribed time using both pairwise comparisons tests and multiple regression models. Neither statistical approach yielded significant differences between the two populations; therefore the data were pooled (within circadian phenotype).

Circadian and ultradian waveform analyses

Lomb–Scargle periodogram (LSP) analyses (described in detail in Prendergast et al., 2012a) were performed to identify the presence or absence of URs and CRs and the number of significant peaks ("complexity") in the UR spectrum (range: 0.3–7.6 h). The level of statistical significance (α) was set to 0.05. Cosinor analyses determined several quantitative measures of URs (range: 0.3–7.6 h) and CRs (range: 22–26 h): robustness (or "prominence," the percentage of variance accounted for by the best-fit cosine model, corresponding to the coefficient of determination R^2 in regression analyses; Refinetti et al., 2007), mesor (rhythm-adjusted mean value around which the waveform oscillates), and amplitude (the difference between the peak or trough value and the mesor), expressed as absolute values (activity counts) and relative amplitude (referenced to the photophase-specific mesor value); the latter incorporates baseline activity levels during each photophase in determining rhythm amplitude and corrects for individual differences in overall activity levels. The level of statistical significance in the cosinor analyses was set to 0.05.

To visualize dynamic changes in CR and UR waveforms, data matrices containing power values generated by LSP analyses were imported into MATLAB (version R2013a, The

MathWorks, Inc., Natick, MA). Time-frequency plots showing frequency (CRs: 22–26 h; URs: 0.3–7.6 h, in 3 min steps) by assessment interval were generated using MATLAB's imagesc function.

Statistical comparisons

The presence or absence of significant CRs and URs between experimental groups was assessed with Chi-square tests. Effects of circadian phenotype and pregnancy on quantitative aspects of CR and UR waveforms were assessed longitudinally using a mixed-models ANOVA (repeated measures, with 2 [ENTR, ARR] × 2 [pregnant, control] independent variables). Pairwise comparisons were performed with two-tailed t-tests, only if a significant omnibus F statistic was obtained. *A priori* planned comparisons were performed without correction for family-wise error. ANOVAs and pairwise comparisons were performed with Statview5.0 (SAS Institute, Cary, NC) and LSP and cosinor analyses with software written by R. Refinetti (available at http://www.circadian.org/softwar.html) (Refinetti et al., 2007). Differences were considered significant if P　0.05. Effect sizes were estimated using partial Eta^2 (η_p^2, for repeated-measures ANOVAs) and Cohen's *d* (for pairwise comparisons, reported as absolute values), using calculations described in Thompson (2006) and the Missouri State University RStats Institute's Cohen's *d* Effect Size Calculator for Independent and Paired Samples (available at www.missouristate.edu/rstats), respectively.

Results

Mating outcomes

18 of 27 ENTR hamsters delivered litters. One ENTR female became circadian arrhythmic after weaning the litter; her data were excluded from all analyses. Among ARR females, 15 of 23 females delivered litters. The incidence of pregnancy did not differ between ENTR and ARR hamsters ($\chi^2 = 0.04$, P > 0.80). Five ARR hamsters transitioned to an ENTR phenotype between mating and weaning; their data likewise were excluded from subsequent analyses.

Circadian rhythms

Representative actograms across the reproductive cycle of dams and over a yoked interval in controls appear in Fig. 1. CRs changed markedly over the course of pregnancy and lactation (Fig. 1A, C) but did not change in non-pregnant controls over the same time span (Fig. 1B, D).

Among females classified as ENTR prior to mating, the proportion of hamsters exhibiting significant CRs decreased markedly during lactation ($\chi^2 = 13.4$, P < 0.001; Fig. 2A). Pregnancy significantly affected CR robustness (pregnancy × time: $F_{5,125} = 3.53$, P < 0.01; $\eta_p^2 = .110$; Fig. 2B) and amplitude (pregnancy × time: $F_{5,125} = 6.90$, P < 0.001; $\eta_p^2 = .178$; Fig. 2C), without significantly altering mesor activity levels (pregnancy: $F_{1,25} = 1.35$, P > 0.20; $\eta_p^2 = .049$; pregnancy × time: $F_{5,125} = 1.38$, P > 0.20; $\eta_p^2 = .050$; Fig. 2D).

Decreases in CR robustness were first evident during late gestation (P < 0.01 vs. pre-mating baseline, $d = 0.80$), with an even more pronounced robustness decline during lactation (P < 0.01 vs. pre-mating baseline;, $d = 1.11$; P < 0.01 vs. controls, $d = 1.16$; Fig. 2B) and a return to values indistinguishable from those of nulliparous controls during the first 10 days of the immediate post-weaning interval (P > 0.80 vs. controls, $d = 0.08$; Fig. 2B).

Decrements in CR amplitude mirrored those for CR robustness: first evident during late gestation, CR amplitude decreased by approximately 50% between mating and early lactation (Fig. 2C; P < 0.01 vs. controls, $d = 1.99$; P < 0.001 vs. pre-mating baseline, $d = 1.72$), and returned to control values during the early post-weaning period (P > 0.80 vs. controls, $d = 0.08$).

By definition, analyses of CR parameters were not possible for ARR hamsters, which exhibited circadian arrhythmia across the entire measurement interval (Supplementary Fig. S1).

Ultradian rhythms — Dark phase

UR prevalence—In ENTR hamsters, dark-phase URs were present in a significantly higher proportion of pregnant than non-pregnant (control) females during late gestation ($\chi^2 = 4.57$, P < 0.05), but not at any other reproductive cycle phase (Fig. 3A). Increases in the incidence of dark-phase URs were also evident in ARR dams during both early ($\chi^2 = 8.17$, P < 0.01; Fig. 3B) and late gestation ($\chi^2 = 10.1$, P < 0.001 relative to pre-mating baseline and compared to values of non-pregnant controls: $\chi^2 = 14.3$, P < 0.001) and were significantly higher than values for ENTR dams (early gestation: $\chi^2 = 18.5$, P < 0.001; late gestation: $\chi^2 = 7.48$, P < 0.01). Dark-phase UR incidence did not differ between pregnant and control hamsters after parturition.

UR complexity—Pregnancy ($F_{1,35} = 8.11$, P < 0.01, $\eta_p^2 = .158$) and circadian phenotype (ARR vs. ENTR, $F_{1,35} = 25.2$, P < 0. 001, $\eta_p^2 = .295$) significantly affected dark-phase UR complexity (Fig. 4A, B). UR complexity was significantly greater in dams than controls during late gestation in ENTR (P < 0.05, $d = 0.82$) and ARR hamsters (P < 0.001, $d = 2.65$). In addition, complexity increased in ARR dams relative to pre-mating values during early gestation (P < 0.05, $d = 1.26$), with additional augmentation during late gestation (P < 0.001, $d = 1.85$; Fig. 4B). Dark-phase UR complexity was greater in pregnant ARR than ENTR hamsters during both early (P < 0.001, $d = 2.31$) and late (P < 0.005, $d = 1.24$) gestation. For both ARR and ENTR hamsters, UR complexity returned to low values during lactation that were comparable to values during the pre-mating interval (P > 0.05, both comparisons; $d = 0.47$, $d = 0.08$, respectively; Fig. 4A, B; Supplementary Fig. S2).

UR period (τ')—Circadian phenotype significantly affected dark phase τ' ($F_{1,36} = 14.3$, P < 0.001, $\eta_p^2 = .221$), but reproductive condition did not ($F_{1,36} = 2.62$, P > 0.10; $\eta_p^2 = .064$; Fig. 4C, D). In ENTR hamsters (Fig. 4C), dark-phase τ' was significantly longer in dams than controls during early gestation (P < 0.01, $d = 1.12$) and lactation (P < 0.05, $d = 0.91$). τ' decreased markedly 1 month post-weaning (P < 0.01 vs. pre-mating for this group, $d = 1.14$; and P < 0.01 vs. controls, $d = 1.25$). Among ARR hamsters, differences between dams and

controls were not evident during any measurement interval (P > 0.05, ds: 0.01–0.73 (range); all comparisons, Fig. 4D). Significant increases in τ' relative to the pre-mating baseline were however evident in ARR dams during early (P < 0.01, $d = 1.37$) and late (P < 0.01, $d = 1.66$) gestation, and during the early post-weaning interval (P < 0.05, $d = 1.40$; Fig. 4D).

UR robustness—Circadian phenotype ($F_{1,36} = 50.1$, P < 0.001, $\eta_p^2 = .368$) and pregnancy ($F_{1,36} = 6.82$, P < 0.05, $\eta_p^2 = .137$) each significantly affected robustness (Fig. 4E, F). Dark-phase robustness was greater in ENTR dams than controls during late gestation and lactation (P < 0.05, both comparisons, $d = 0.91$, $d = 0.80$, respectively), but not thereafter (P > 0.05, both comparisons; $d = 0.76$, $d = 0.42$, respectively). In ARR hamsters, increases in robustness in dams relative to controls were evident in late gestation only (P < 0.01, $d = 1.60$; Fig. 4F). Within-group increases in dark-phase τ' (relative to pre-mating values) were first evident in ENTR hamsters during late gestation (P < 0.05, $d = 0.62$), and first appeared in ARR hamsters during early gestation (P < 0.05, $d = 0.78$).

UR amplitude—Circadian phenotype markedly affected dark-phase amplitude ($F_{1,30} = 133.7$, P < 0.001, $\eta_p^2 = .450$); the main effect of pregnancy on this measure fell just short of statistical significance ($F_{1,30} = 3.73$, P = 0.06, $\eta_p^2 = .100$) but pregnancy significantly affected the pattern of change in amplitude over time (pregnancy × time interaction: $F_{5,150} = 2.45$, P < 0.05; $\eta_p^2 = .070$; Fig. 4G, H). In ENTR hamsters, dark-phase UR amplitude was significantly greater in dams than controls during early gestation (P < 0.05, $d = .085$) and lactation (P < 0.001, $d = 1.49$), but not during any other interval. UR amplitude did not differ between ARR dams and ARR controls at any stage of the reproductive cycle (P > 0.05, ds: 0.03–0.66 (range); all comparisons Fig. 4H). Dark-phase amplitude was greater in ARR than ENTR dams during each interval (P < 0.001, ds: 2.11–4.86 (range); all comparisons) except lactation (P > 0.05, $d = 0.74$).

UR mesor—Dark-phase mesor activity levels were greater in ENTR than ARR hamsters ($F_{1,30} = 21.7$, P < 0.001, $\eta_p^2 = .296$), but no significant overall modulation by pregnancy was evident ($F_{1,30} = 0.03$, P > 0.80; $\eta_p^2 = .001$; Supplementary Fig. S3).

Ultradian rhythms — Light phase

UR prevalence—Light-phase URs were present in approximately 50% of control ENTR hamsters at any point of the experiment and increased significantly in pregnant ENTR females during late gestation (relative to the pre-mating interval: $\chi^2 = 4.27$, P < 0.05; also compared to non-pregnant controls: $\chi^2 = 4.3$, P < 0.05), and again during the early post-weaning interval ($\chi^2 = 4.73$, P < 0.05), but returned to normal levels thereafter (1 month post-weaning: $\chi^2 = 1.10$, P > 0.20; Fig. 5A). Light-phase UR incidence increased between the pre-mating interval and late gestation in pregnant ARR females ($\chi^2 = 4.55$, P < 0.05; Fig. 5B), and light-phase URs were detected less often in pregnant ARR than pregnant ENTR females during the immediate post-weaning interval ($\chi^2 = 7.99$, P < 0.01).

UR complexity—No main effect of circadian phenotype was evident, but pregnancy altered the pattern of change in complexity over time (pregnancy × time interaction: $F_{5,175} = 3.07$, P = 0.01; $\eta_p^2 = .075$; Fig. 6A, B; Supplementary Fig. S2). In ENTR dams, increases in UR complexity emerged during late gestation (P < 0.01, $d = 1.13$), disappeared during lactation (P > 0.05, $d = 0.20$), only to re-emerge during the early post-weaning interval (P < 0.05, $d = 0.83$). The apparent increases in complexity during late gestation in ARR dams fell short of statistical significance (P = 0.07, $d = 0.94$).

UR period—Pregnancy altered the pattern of change in τ' over time (pregnancy × time interaction: $F_{5,180} = 2.27$, P < 0.05; $\eta_p^2 = .056$; Fig. 6C, D), but circadian phenotype was without effect. Significant τ' increases emerged during late gestation in both ENTR (P < 0.05 vs. controls, $d = 0.85$) and ARR (P < 0.05 vs. pre-mating value, $d = 1.31$).

UR robustness—Robustness was comparable in ENTR and ARR hamsters ($F_{1,36} < 0.01$, P > 0.90, $\eta_p^2 = .001$), but pregnancy markedly affected the change over time (pregnancy × time interaction: $F_{5,180} = 3.66$, P < 0.005; $\eta_p^2 = .084$; Fig. 6E, F). URs were more robust in ENTR and ARR dams than in respective controls during late gestation (P < 0.001, $d = 1.48$; and P < 0.05, $d = 1.17$; respectively) and did not differ between ARR and ENTR dams during any reproductive interval.

UR amplitude—Relative amplitude of the waveform did not differ between ENTR and ARR hamsters ($F_{5,170} = 1.27$, P > 0.20, $\eta_p^2 = .035$), nor were systematic differences evident between pregnant and non-pregnant hamsters over time ($F_{5,170} = 0.15$, P > 0.90; $\eta_p^2 = .004$; Fig. 6G, H).

UR mesor—Neither circadian phenotype ($F_{5,175} = 1.86(1.01)$, P > 0.10 (P > 0.40), $\eta_p^2 = .027$) nor pregnancy status ($F_{5,175} = 1.01(0.60)$, P > 0.40 (P > 0.50), $\eta_p^2 = .017$) affected the pattern of change in UR mesor (Supplementary Fig. S3).

Relations between pregnancy-induced changes in the CR and UR waveforms

Among ENTR dams, no significant linear relation was evident between changes from pre-mating to late gestation in CR robustness and dark-phase UR robustness ($R^2 = 0.094$, P > 0.20; not illustrated); the regression between change in CR robustness and light-phase UR robustness likewise was not significant ($R^2 < 0.01$, P > 0.80; not illustrated).

Discussion

Progressive decrements in circadian robustness and amplitude over the course of gestation were accompanied by enhanced ultradian rhythms in circadian-entrained dams. Dark-phase UR period and amplitude increased during early gestation, and complexity and robustness were greater during late gestation. UR complexity, period, and robustness of pregnant dams were also greater than those of non-pregnant controls during late gestation. During lactation, circadian arrhythmia was pronounced and light-phase URs were largely absent; dark-phase URs, on the other hand, were robust and increased in amplitude. Most pregnancy-associated

enhancements of URs also were observed in arrhythmic dams lacking functional circadian organization (ARR hamsters). This extends previous observations in which CR decrements and UR enhancements occurred contemporaneously over the course of the reproductive cycle in Syrian hamsters with normal circadian organization housed in short day lengths (Prendergast et al., 2012a). Per partum changes in temporal patterning of behavior have been documented in diverse taxa (Bloch et al., 2013). The pregnancy-associated enhancements of URs in ARR hamsters indicate that pregnancy effects on the UR waveform, whether neurally or hormonally mediated, are not dependent on coherent circadian organization.

The mere absence of CRs in ARR hamsters did not enhance most aspects of URs. With the exception of dark-phase UR robustness (Fig. 4E, F), which was greater in ARR hamsters, UR parameters were comparable in ARR and ENTR hamsters prior to mating. This finding is for the most part consistent with a recent report indicating that the DPS-induced ARR phenotype does not, by itself, augment UR incidence or waveforms (Prendergast et al., 2012b). Therefore, the influence of the circadian system on URs was examined by assessing whether pregnancy-induced enhancements were similar in ENTR and ARR dams. If URs were not enhanced in ARR dams, one could conclude that functional circadian organization is required for gestation to influence UR generation. In contrast, the augmentation of URs in ARR dams would suggest that gestational enhancements in URs are not dependent on changes in the circadian system. The results are consistent with the second scenario. During gestation, URs of ARR dams were more prevalent, complex, and robust than those of non-pregnant ARR controls. The only quantitative metric of dark-phase URs exhibited by ENTR but not by ARR dams was the increase in dark-phase UR amplitude. Strong similarities were also evident between ENTR and ARR dams in light-phase URs, with significant increases in period length, robustness, and incidence occurring in both circadian phenotypes.

Enhancement and suppression of URs and CRs, respectively, during pregnancy may be influenced by changes in ovarian and pituitary hormone secretion. URs and CRs change markedly over the 4-day estrous cycle in Syrian hamsters (Prendergast et al., 2012a), and hamster CRs are modulated by ovarian hormones (Morin et al., 1977; Takahashi and Menaker, 1980).

The increased incidence of dark-phase URs appeared more rapidly in ARR than ENTR dams. Throughout gestation, the percentage of dams with dark-phase URs was significantly greater in the ARR group. Gestational increases in dark-phase UR complexity and robustness also emerged earlier and were greater in ARR than ENTR dams. The absence of coherent CRs may be causally related to these effects on URs.

During lactation, CRs of ENTR dams were markedly reduced in prevalence and amplitude. The light-phase UR waveform also was diminished; however, the period of dark-phase URs was longer and robustness and amplitude were greater in ENTR dams than unmated controls. Post-partum changes in the temporal patterning of behavior have been observed throughout the animal kingdom. Human mothers caring for newborns exhibit weak CRs during the first 3 postpartum weeks; CRs subsequently gain prominence, as rest–activity patterns of infants and mothers synchronize (Nishihara et al., 2002). The persistence of robust dark-phase URs in early post-partum Siberian hamsters differs from the markedly

inhibited dark-phase URs in lactating Syrian hamsters (Prendergast et al., 2012a). The significance of this species difference remains unclear.

Upon weaning, the CR waveform of Siberian hamster dams exhibited near-complete recovery to pre-mating baseline values. A similarly rapid rebound to control values was noted previously in Syrian hamsters, whose pregnancy-induced enhancements of URs were restored to lower values immediately after weaning (Prendergast et al., 2012a). Syrian hamsters in the latter study were maintained in a short day length (10 h L:14 h D). Circadian entrainment to short photoperiods decreases the amplitude of CRs (Evans et al., 2003; Pittendrigh et al., 1984; Prendergast and Zucker, 2012), perhaps accounting for reduced CR and UR amplitudes of short-day Syrian compared to long-day Siberian females. The rapidity of CR recovery after weaning suggests that CR decrements during lactation reflect negative masking by the demands of the litter, rather than changes in the circadian pacemaker. Evaluation of phase-response curves to light and molecular responses of the SCN to light in lactating dams will be required to resolve this issue. Persistence of enhanced light-phase UR waveforms for a week after removal of offspring from the nest suggests that physiological changes associated with pregnancy and lactation can persist, albeit for less than a month, after weaning of the litter.

Dams of altricial species actively care for their young. During the first few weeks after birth, pups remain in close physical proximity to their mothers who provide milk, stimulate urination and defecation by licking the pups' anogenital areas, and regulate pup body temperature (Madison, 1978). The quality and quantity of maternal care is essential for normal development of offspring (Fodor et al., 2012; Fonken et al., 2011). By abandoning obligate circadian structure in behavior, dams may incur fitness benefits that permit more efficient interactions with circadian arrhythmic pups. Bloch et al. (2013) note that when circadian organization may not be critical, ultradian organization could also allow temporal coordination. Evidence from invertebrates with high maternal investment suggests that non-circadian activity may contribute to fecundity and reproductive success (Eban-Rothschild et al., 2011). In addition, changes in human URs of systolic arterial pressure have been reported in a subset of women during the second and third trimesters (Stoynev et al., 1999).

In summary, the present data reaffirm and extend earlier work (Albers et al., 1981; Prendergast et al., 2012a) documenting modulation of circadian and ultradian rhythms during pregnancy and lactation. Enhanced light- and dark-phase URs during the reproductive cycle occur in the absence of coherent circadian organization; the relaxation of circadian control facilitates enhancement of ultradian rhythms during select phases of the reproductive cycle.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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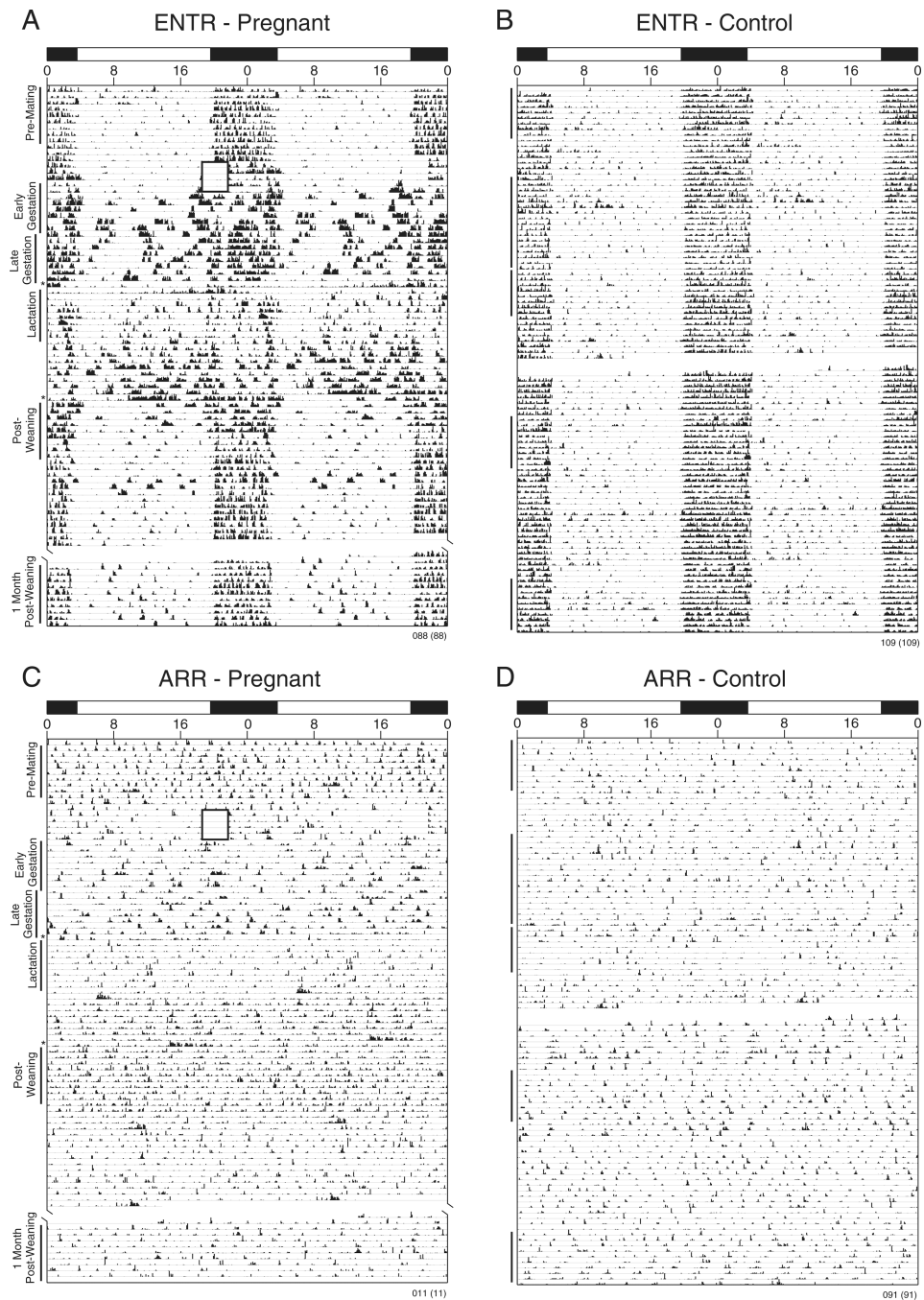
Fig. 1.
Circadian locomotor activity over successive stages of the reproductive cycle. Representative double-plotted activity records of pregnant (panels A and C) and control (non-pregnant; panels B and D) hamsters. Prior to mating (pre-mating), hamsters in panels A and B exhibited the ENTR phenotype, and hamsters in panels C and D exhibited the ARR phenotype. Clock time is indicated on the horizontal axis at the top of each actogram, along with light (white) and dark (black) phases of the 16 L:8D photocycle. In panels A and C, boxes indicate the 5 successive nights during which females were housed with males for

mating, and asterisks along the ordinate axis indicate the days of parturition and weaning. Intervals subjected to data analysis are indicated to the left of panels A and C: Pre-mating = 10 days, prior to mating; Early Gestation = gestational days 0–8; Late Gestation = gestational days 9–17; Lactation = postnatal days 0–9; Post-weaning = postnatal days 19–28; 1 Month post-weaning = postnatal days 49–58. Yoked intervals used for controls are indicated with vertical bars along the ordinates of panels B and D.
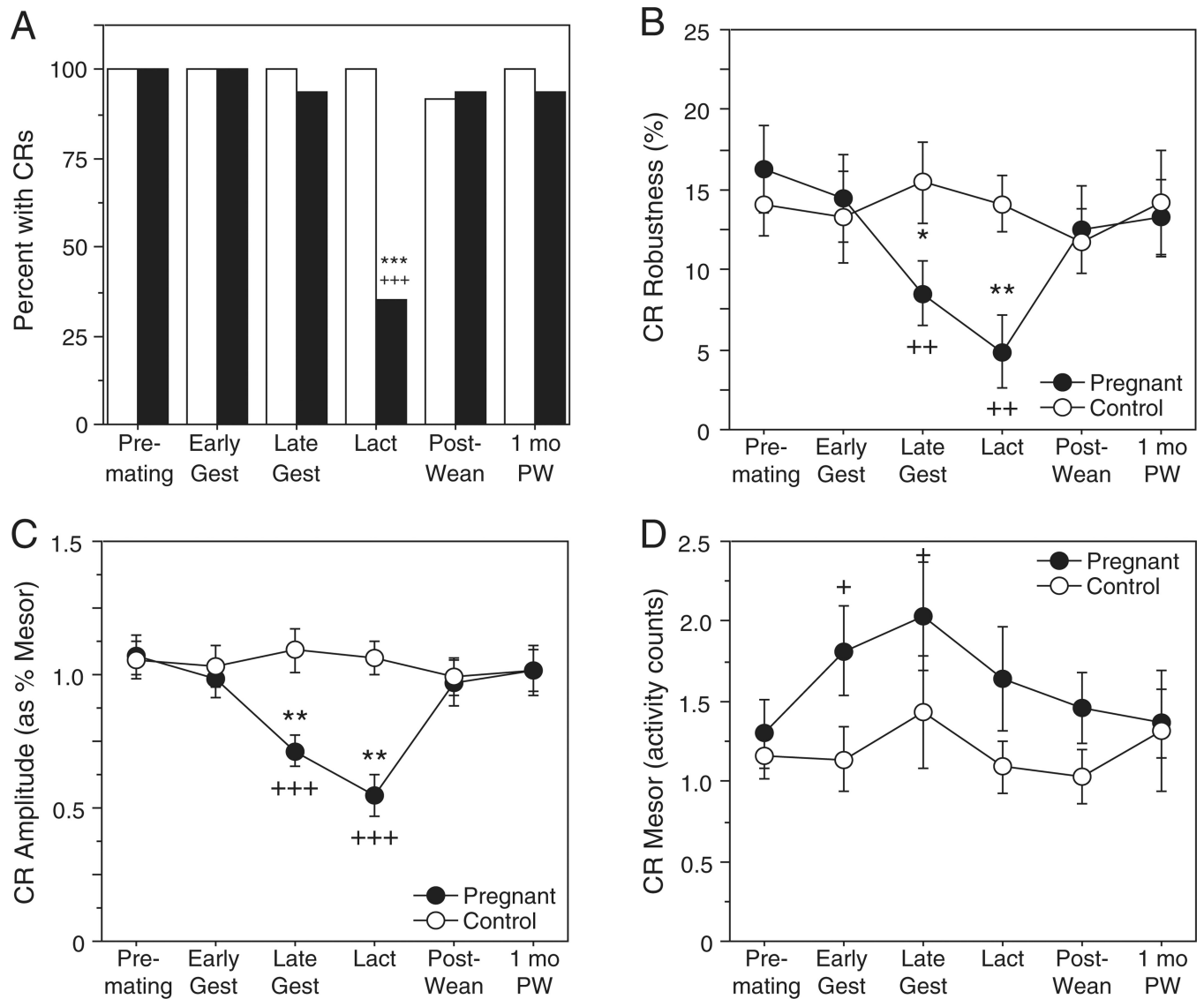
Fig. 2.
Pregnancy and lactation decrease circadian power. (A) Percent of ENTR hamsters expressing significant circadian rhythms (CRs) as determined by LSP. Black and white bars designate pregnant and control females, respectively. (B) Mean ± SEM CR robustness, (C) relative CR amplitude and (D) mesor activity levels of ENTR dams and controls. *P < 0.05, **P < 0.01 vs. control value, at specified interval. +P < 0.05, ++P < 0.01, +++P < 0.001 vs. pre-mating value, within group.
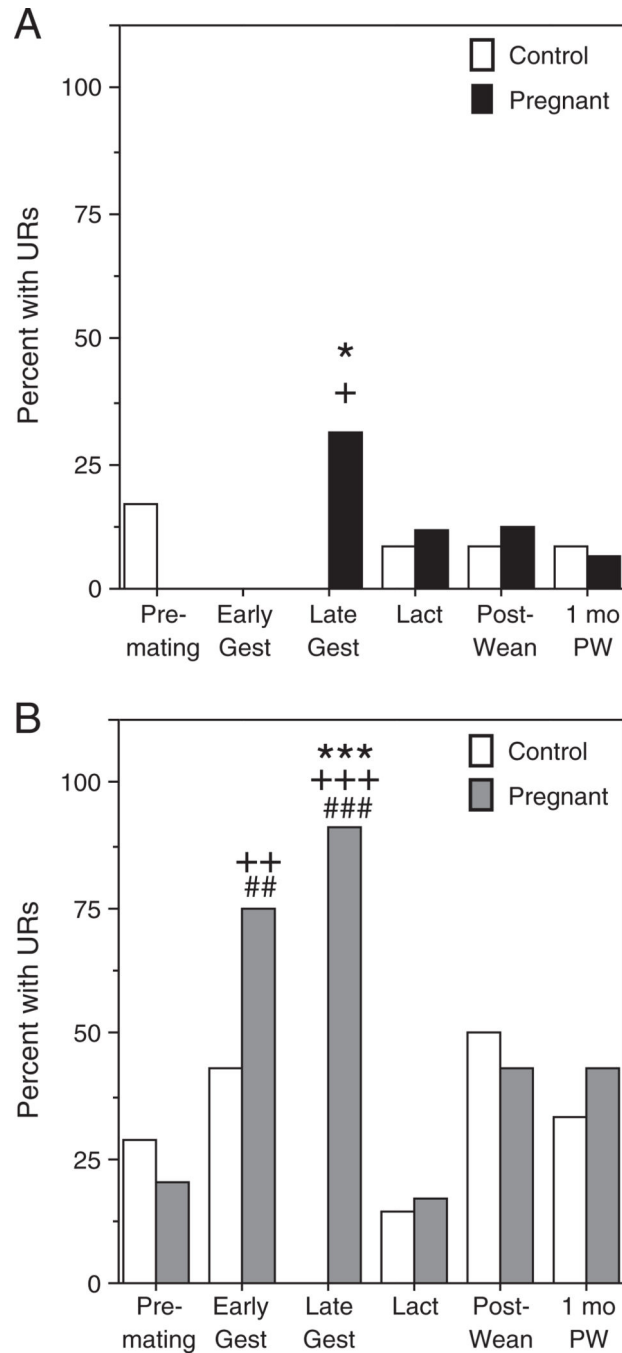
Fig. 3.
Enhancement of dark-phase URs during pregnancy occurs in both ENTR and ARR hamsters. Percent of (A) ENTR hamsters and (B) ARR hamsters expressing significant dark-phase URs as determined by LSP analysis. *P < 0.05, ***P < 0.001 vs. control value, at specified interval. ##P < 0.01, ###P < 0.001 vs. ARR value, within reproductive group, at specified time point. +P < 0.05, ++P < 0.01, +++P < 0.001 vs. pre-mating value, within group.
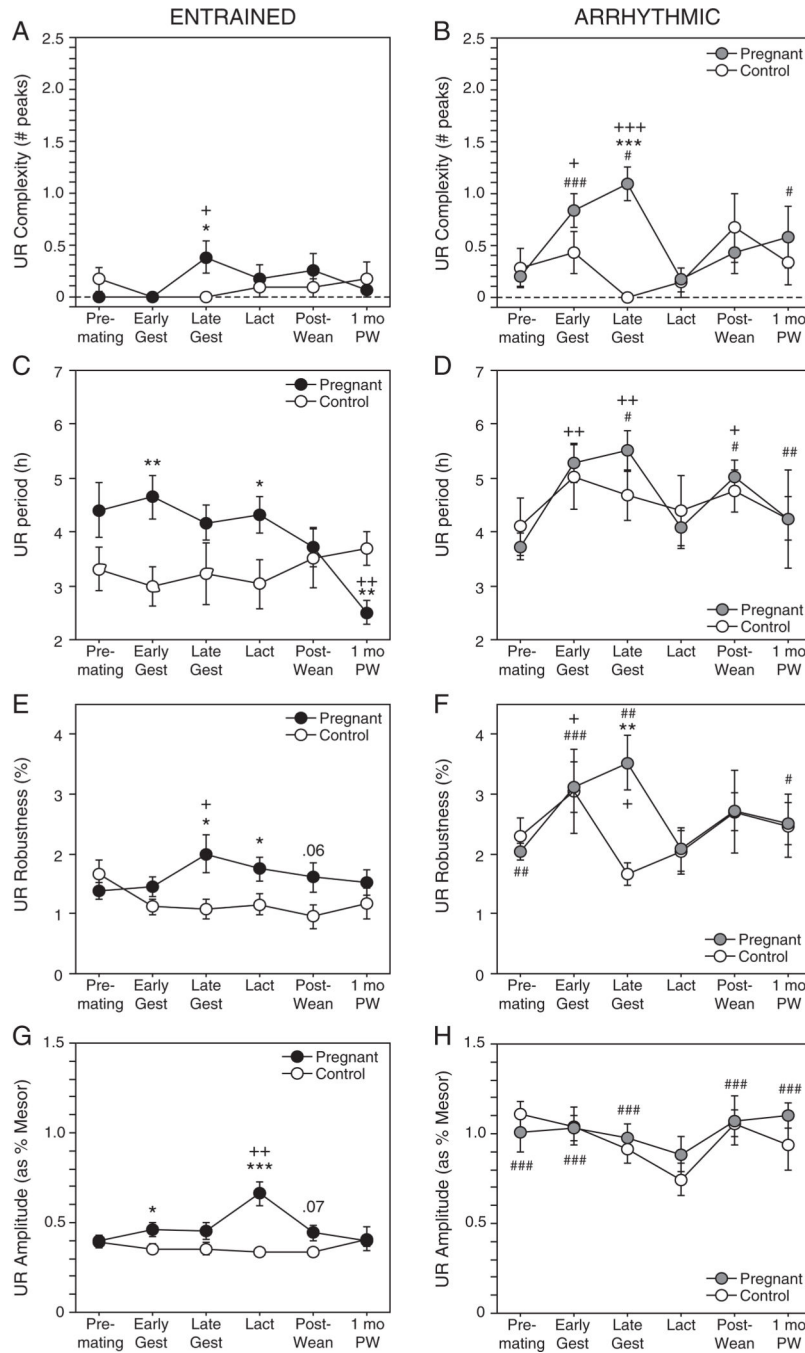
Fig. 4.
Enhancement of dark-phase URs during pregnancy in ENTR and ARR hamsters. Mean ± SEM UR complexity (A), period (C), robustness (E), and relative amplitude (G) in ENTR dams and non-pregnant controls, and UR complexity (B), period (D), robustness (F), and relative amplitude (H) in ARR dams and non-pregnant ARR controls. *P < 0.05, **P < 0.01, ***P < 0.001 vs. control value, at specified interval. #P < 0.05, ##P < 0.01, ###P < 0.001 vs. ARR value, within reproductive group, at specified time point. +P < 0.05, ++P < 0.01, +++P < 0.001 vs. pre-mating value, within group.
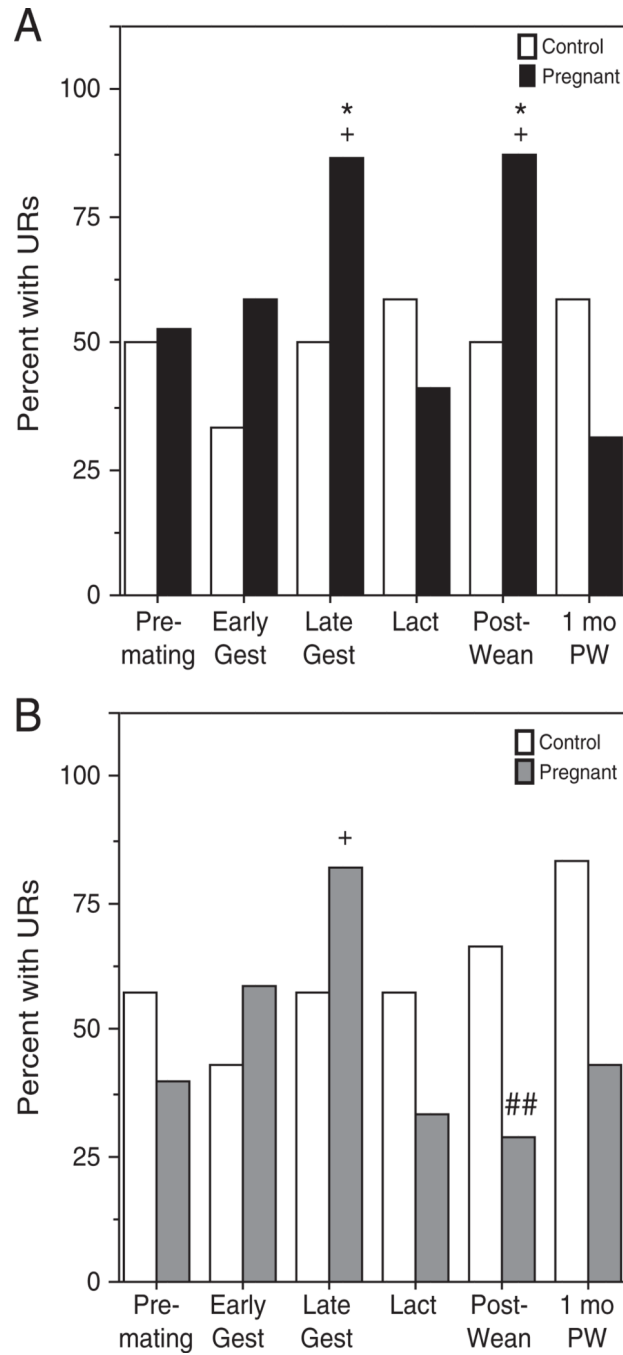
Fig. 5.
Enhancement of light-phase URs during pregnancy persists in ARR hamsters. Percent of (A) ENTR hamsters and (B) ARR hamsters expressing significant light-phase URs as determined by LSP analysis. *P < 0.05 vs. control value, at specified interval. ##P < 0.01 vs. ARR value, within reproductive group, at specified time point. +P < 0.05 vs. pre-mating value, within group.
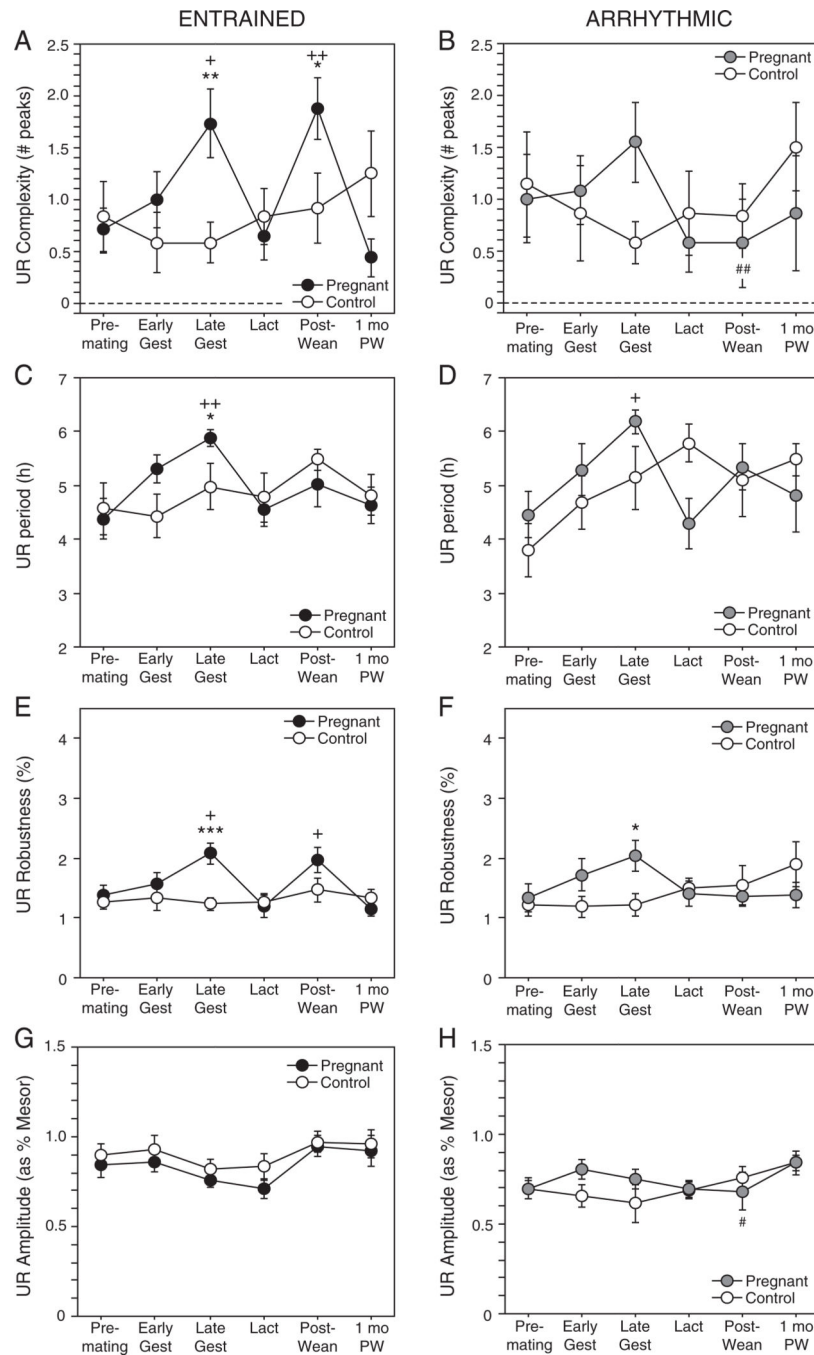
Fig. 6.

Enhancement of light-phase URs during pregnancy in ENTR and ARR hamsters. Mean ± SEM UR complexity (A), period (C), robustness (E) and relative amplitude (G) in ENTR dams and non-pregnant controls and UR complexity (B), period (D), robustness (F), and relative amplitude (H) in ARR dams and non-pregnant ARR controls. *P < 0.05, **P < 0.01, ***P < 0.001 vs. control value, at specified interval. #P < 0.05, ##P < 0.01 vs. ARR value,

within reproductive group, at specified time point. +P < 0.05, ++P < 0.01 vs. pre-mating value, within group.