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Effect of arousing stimuli on circulating corticosterone and the circadian rhythms of luteinizing hormone (LH) surges and locomotor activity in estradiol-treated ovariectomized (ovx+EB) Syrian hamsters

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Abstract

In most proestrous hamsters, novel wheel exposure phase advances activity rhythms and blocks the preovulatory LH surge, which occurs 2 h earlier the next day. Because wheel immobilization does not prevent these effects we hypothesized that arousal alone blocks and phase advances the LH surge. Ovariectomized (ovx) hamsters received a jugular vein cannula and estradiol benzoate (EB) or vehicle was injected sc. The next day (Day 1), at zeitgeber time (ZT) 4-5 (ZT 12=lights off), after obtaining a blood sample, each hamster was exposed to constant darkness (DD), and either remained in her home cage or was transferred to a new cage and exposed to a running wheel or a 2-h arousal paradigm. Blood samples were obtained in dim red light and activity was recorded hourly until ~ZT 10-11 on Days 1 and 2. For the next 1-2 weeks, activity was monitored in DD. Plasma LH and corticosterone were assessed by RIA. Novel wheel exposure or arousal at ZT 4 greatly attenuated the Day 1 LH surge in ovx+EB hamsters, and phase advanced the Day 2 LH surge by about 2 hours. In proestrous hamsters, novel wheel exposure led to a prolonged (>2 h) increase in corticosterone levels only when LH surges were blocked. Phase advances in activity rhythms were enhanced by estradiol and arousal. The results suggest that estradiol modulates the effectiveness of non-photic stimuli. The role of the increased activity of the hypothalamic-pituitary-adrenal axis associated with novel wheel-induced attenuation of LH surges in ovx+EB hamsters remains to be determined.

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Disclosures

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Keywords

Circadian rhythm; corticosterone; luteinizing hormone; novel wheel; Syrian hamster; arousal; locomotor activity rhythm; phase advance; estradiol benzoate; ovariectomy

Introduction

Coordination of endogenously generated 24-h (circadian) biological rhythms within the body and with environmental timing signals is essential for health and well-being. When circadian rhythms are chronically disrupted or desynchronized, as in shift workers, there is a greater incidence of metabolic disorders, cognitive impairment, depression, infertility and several cancers, including breast and prostate (Hastings et al., 2003; Spiegel et al., 2009; Turek, 2007; Moser et al., 2006; Davidson et al., 2006; Davis and Mirick, 2006). Changes in the relative timing of circadian rhythms, i.e., phase shifts, are caused not only by changes in the light:dark cycle, but also by a variety of non-photic signals. The role of non-photic signals in inducing phase shifts in circadian activity rhythms has been well-studied in male rodents, but much less so in females. Non-photic signals that have been investigated in male rodents consist of transfer to a new environment (Mrosovsky, 1988), confinement to a new wheel (Reebs and Mrosovsky, 1989), sleep deprivation, dark pulses, benzodiazepines, or social interaction (Mrosovsky, 1996). In female Syrian hamsters, only exposure to a novel running wheel has been studied, and it was found to phase advance the circadian rhythm of locomotor activity, and to disrupt a neuroendocrine rhythm, the luteinizing hormone (LH) surge (Legan et al., 2010; Duncan et al., 2014).

In laboratory rodents, the luteinizing hormone (LH) surge is controlled by the circadian timing system such that it always occurs at the same time of day, a characteristic most clearly demonstrated in estradiol-treated ovariectomized animals, which exhibit daily LH surges (Norman et al., 1973; Legan and Karsch, 1975; Christian et al., 2005). In ovary-intact Syrian hamsters the preovulatory LH surge occurs every 4 days on proestrus, and peaks on the average at Zeitgeber time (ZT) 9, which is 3 hours before lights off (= ZT 12) (Stetson and Watson-Whitmyre, 1977; Duncan et al., 2014). Exposure of proestrous Syrian hamsters to a novel cage and wheel at ZT 5 blocks the LH surge that afternoon in most hamsters, which results in a 1-day delay of the LH surge and thereby a 1-day prolongation of the estrous cycle (Legan et al., 2010). When the LH surge occurs on the next day, it is phase-advanced by about 2 hours, peaking on the average at ZT 7. In addition, the circadian rhythm in locomotor activity in all novel wheel-exposed proestrous hamsters is phase-advanced by about 2 hours indicating that exposure to a new cage and novel wheel at ZT 5 on proestrus phase advances the master circadian pacemaker (Legan et al., 2010).

Similar phase advances in the circadian activity rhythm in male hamsters exposed to a variety of nonphotic stimuli have been well-documented (Mrosovsky, 1988; Reebs and Mrosovsky, 1989; Mrosovsky, 1996). Because the phase shifts in the activity rhythm are often correlated with the amount of physical activity and can be blocked by restraint (Van Reeth and Turek, 1989; Wickland and Turek, 1991), it was postulated that locomotor activity is an essential part of the signal that caused the phase advances (Mrosovsky, 1996).

A subsequent finding that sleep deprivation by gentle handling induces similar phase advances in the activity rhythm demonstrated that locomotor activity is not essential and suggested that arousal or wakefulness is the critical stimulus for phase shifting the master circadian oscillator (Antle and Mistlberger, 2000). (It should be noted that herein, the term “arousal” refers to wakefulness, stimulation to action, or physiological readiness for activity (www.merriam-webster.com/dictionary/arousal). However, other methods for maintaining arousal or wakefulness, such as restraint stress, confinement to a platform over water (Mistlberger et al., 2003) or injections of caffeine (Antle et al., 2001), yohimbine or modanafil (Webb et al., 2006), do not induce phase shifts, indicating that all types of arousal do not have equivalent effects on the circadian timing system.

Some non-phototic stimuli are associated with glucocorticoid release, and indeed circulating glucocorticoid levels increase acutely in male rats following transfer to a new cage and/or exposure to a novel wheel (Reul et al., 1993; Seggie and Brown, 1975; Baldwin et al., 1974; Buijs et al., 1997), two stimuli which induce phase advances in the circadian activity rhythm. In contrast, arousal associated with excessive elevations in circulating glucocorticoids may block the phase advance. Thus in male hamsters, stress-loaded restraint, which elicits a 5-fold increase in circulating cortisol levels, does not cause phase advances in locomotor activity, whereas confinement to a wheel, which does not alter cortisol levels based on samples taken 30, 90 and 180 min later, induces large phase advances in locomotor activity (Mistlberger et al., 2003). Thus it is not clear what role circulating glucocorticoid levels play in non-phototic phase resetting. In female rats, with respect to LH surges, acute administration of glucocorticoids can block estradiol-induced LH surges (Baldwin, 1979; Gore et al., 2006). However, whether exposure to a novel wheel induces an increase in circulating glucocorticoids and whether this is sufficient to block the LH surge remains to be determined. In this regard it should be noted that there are species and sex differences in the stress axis that may alter the responsiveness of the circadian system to non-phototic stimuli. Thus, basal circulating levels of stress hormones are higher and the response to stress is greater in male than in female hamsters (Gaskin and Kitay, 1970; Weinberg and Wong, 1986), which is the reverse of the sex differences in rats (Atkinson and Waddell, 1997; Viau et al., 2005).

Based on the foregoing considerations, the following studies were performed to test two hypotheses: (1) that a non-specific arousing stimulus would induce a phase advance in locomotor activity and block the LH surge, similar to the effect of exposure to a novel wheel, and (2) that in those hamsters whose LH surge was blocked, the arousing stimulus would induce a greater increase in circulating corticosterone concentrations. Estradiol-treated ovariectomized (ovx+EB) hamsters were used to test the first hypothesis because they exhibit daily LH surges, in contrast to every 4th day as in ovary-intact females, thus providing a more efficient experimental animal model for study of the mechanisms controlling the LH surge (Norman et al., 1973). We first confirmed the occurrence of 2 consecutive daily LH surges under conditions of wheel running and DD, and determined the time of the LH peak in ovx+EB hamsters. Next, we determined whether exposure to a novel wheel has the same phase-shifting effects on the circadian rhythms in the LH surge and locomotor activity as in ovary-intact hamsters. Third, a paradigm of sequential presentation of novel objects was substituted for novel wheel exposure in order to test the hypothesis that

a non-specific arousing stimulus can block and phase advance the LH surge in ovx+EB hamsters. Finally, to test the second hypothesis, that blockade of the LH surge is associated with a greater increase in circulating corticosterone, we investigated the acute glucocorticoid response in ovary-intact proestrous hamsters exposed to a novel running wheel.

Materials and Methods

Animals

All the following procedures were performed with the approval of the University of Kentucky Institutional Animal Care and Use Committee according to the standards of the Institute of Laboratory Animal Resources established in the Guide for Care and Use of Laboratory Animals, 8th edition. Adult female Syrian hamsters were housed individually under a 14L:10D photoperiod in a temperature and humidity-controlled environment with ad lib access to food and water. Throughout the study, either a male hamster or soiled male hamster bedding (changed weekly) was located within several feet of each female hamster's cage. To establish that the females were having regular estrous cycles, estrous discharges were evaluated daily as described previously (Orsini, 1961; Legan et al., 2009) until 3 consecutive estrous discharges were observed at 4-day intervals, and afterwards they were inspected every 4th day on the expected day of estrus. Once regular estrous cyclicity was established, the hamsters were placed in photoperiod- and temperature-controlled, ventilated compartments.

Activity rhythm monitoring and analysis

Home cage activity was recorded continuously for at least 2 weeks before cannulation surgery, with either a running wheel (Expt. 1) or an infrared motion detector (Expts. 2-4) interfaced with a computer to Clocklab software (Coulbourn Instruments, Whitehall, PA). During experiments, home cage or running wheel activity was recorded continuously except during the two days when blood samples were collected. Phase shifts in the circadian activity rhythms were determined using the linear regression method and Clocklab software, as described previously (Legan et al., 2009).

Experiment 1, Daily LH Surges in OVX+EB Hamsters

At least 1 week after receiving running wheels, hamsters were bilaterally ovariectomized (ovx) on random days of their estrous cycles. Fifteen days later, the hamsters were anesthetized with isoflurane and surgically fitted with a right jugular cannula for blood collection and estradiol benzoate (EB, 50 µg in corn oil) was injected s.c. at the end of the surgery. The animals were returned to the animal facility overnight, and the next morning a blood sample (0.3 ml) was obtained in the facility at ZT 3 (ZT 12 = lights off). About 10-15 min before ZT 5, hamsters were isolated in their home cages by covering them with a drape or placing them inside transport boxes. They were then transported on a lab cart from the basement animal facility to the 6th floor laboratory in the same building. After a second blood sample was obtained at ZT 5, the hamsters were immediately exposed to constant darkness (DD) in their home cages. Additional blood samples for determination of LH surges were obtained hourly from ZT 6 – 10 in dim red light. The animals remained in the laboratory overnight, and the same blood sampling schedule was followed on Day 2, but at a

lower volume (0.25 ml). Following each collection, the blood samples were centrifuged and the plasma was drawn off and stored at -20 C until assayed. The red blood cells were resuspended in an equal volume of heparinized saline and infused via the cannula after the next sample was withdrawn, followed by heparinized saline. During the 2 sampling days, the number of wheel revolutions was monitored and recorded hourly between ZT 5 and ZT 10 using an automated event counter. After collection of the last sample on Day 2, the hamsters were anesthetized with isoflurane, and their cannulae were trimmed, knotted and interiorized. Thereafter the hamsters were returned to the animal facility where they remained in DD and running activity was recorded for at least 7 more days.

Experiment 2, Comparison of the effect of a novel wheel on daily LH surges and circadian phase shifts in ovx+oil, ovx+EB and intact proestrous hamsters

Thirteen or 14 days after ovx, two groups of hamsters were fitted with right jugular cannulae and at the end of surgery, were injected with either estradiol benzoate (50 µg EB, ovx+EB) or corn oil vehicle (ovx+oil) s.c., and later returned to the animal facility. A group of ovary-intact animals was also fitted with jugular cannulae on the day before proestrus (diestrus 2). On the day after cannulation, animals from all 3 groups were transferred to the laboratory where blood samples were collected hourly for 2 days for determination of LH surges. The intact and ovx+oil groups were brought to the laboratory shortly before ZT 5. At ZT 5, a blood sample was obtained, after which the animals were transferred to a new cage with a running wheel and exposed to DD. The ovx+EB group was treated identically except that they were brought to the laboratory and sampling was begun one hour earlier, at ZT 4. Hourly blood samples were obtained for the next 6 h from all animals for the next 2 days and the number of wheel revolutions was monitored and recorded hourly as described in Experiment 1. In addition, the intact animals were checked for presence of an estrous discharge. Blood samples were processed and red blood cells resuspended and re-injected as in Experiment 1. After the last sample was obtained, the cannulae were interiorized as in Experiment 1 and the animals were returned to the animal facility for subsequent monitoring of running activity in DD for at least 7 days.

Experiment 3, Can arousing stimuli other than a novel wheel block and phase advance the LH surge and the circadian activity rhythm?

Hamsters were treated the same as those in Experiment 1 until 13-14 days after ovariectomy, when they were fitted with a right jugular cannula and all were injected with EB. On the next day shortly before ZT 5, they were transported to the laboratory. After collection of a blood sample at ZT 5, they were exposed to dim red light and were either returned to their home cages or were transferred to a new cage and subjected to an arousal paradigm for the next 2 hours. To maintain arousal, four different stimuli were presented for 30 minutes each in the following order: a second con-specific female hamster placed on the other side of a screen that was first inserted to divide the cage in half; an acrylic hamster tunnel tube; a toy igloo; and a different conspecific hamster separated by the screen, which was washed between uses. The screen was only used for separating the experimental and conspecific test hamsters to prevent potential aggressive behavior. Each stimulus was removed as the next item was placed in the cage. Throughout the 2-hour period, hamster behavior was observed. Whenever necessary, sleeping was prevented by tapping on the cage, gentle puffs of air, or

lifting the cage lid momentarily. Behavior/activity was recorded at least every 15 minutes or whenever it changed. Blood samples were obtained hourly for the next 6 hours, and at the same times the next day, and all samples were processed as described in Experiment 1. After the last sample was withdrawn, the cannulae were interiorized as described in Experiment 1 and the animals were returned to the animal facility in DD for about 2 weeks for determination of phase shifts.

Because the LH surge occurred one hour earlier in ovx+EB hamsters, the experiment was repeated with the arousal paradigm initiated at ZT 4. In the repeat experiment, an additional toy was used instead of conspecific hamsters. The arousing stimuli, which were presented consecutively every 30 minutes in the following order, were: insertion of a screen that divided the cage in half; a whiffle ball; a hamster tunnel tube; and a toy igloo. Each item was removed at the time of placing a new item in the cage. Blood samples were obtained from ZT 4-10 on 2 consecutive days, and were processed as in the previous experiments. After collection of the last sample on Day 2, the cannulae were interiorized and activity rhythms in DD were monitored as described above.

Experiment 4, Is blockade of the LH surge associated with a greater increase in plasma corticosterone?

A group of ovx+EB hamsters and 2 groups of intact proestrous hamsters that either remained in their home cage or were exposed to a novel wheel were treated the same as those in similar groups in Experiment 2 with the following differences. Blood samples were only collected on one day after cannulation, and in addition to the hourly samples obtained from ZT 5-11, two additional blood samples were obtained at 15 and 30 min after the first sample. Plasma corticosterone levels were determined in all plasma samples and LH concentrations were determined only in the hourly samples. Although hamsters secrete both cortisol and corticosterone, both of which exhibit diurnal variations and increase with stress (Albers et al., 1985), cortisol concentrations were not determined due to limited sample volumes.

LH RIA

Plasma LH concentrations (ng rLH-RP-3/ml) were determined by a radioimmunoassay described previously (Legan et al., 2009). The standard and the purified LH for iodination were obtained from Dr. A. Parlow at the National Hormone and Peptide Program. The primary antibody, CSU120, obtained from Dr. Terry Nett (Colorado State University, Fort Collins, CO), was used at a dilution of 1:10,000. The samples were analyzed in 16 assays for which the sensitivity averaged 0.018 ± 0.001 ng/tube. Intra- and inter-assay coefficients of variation (CVs) were determined for two standard sera that were analyzed at the beginning, middle and end of every assay for quality control purposes. One standard serum pool consisted of intact female hamster serum containing 1.18 ng LH/ml ($n=7$) that inhibited binding to 60% of $B_{\max}$ and had intra- and inter-assay CVs in these 16 assays of 5.7% and 11.0%, respectively. The other standard serum pool consisted of ovariectomized hamster serum containing 7.72 ng LH/ml ($n=5$) that inhibited binding to 31% of $B_{\max}$ and had intra- and inter-assay CVs of 8.0% and 14.7%, respectively.

Corticosterone RIA

Serum samples were assayed in duplicate using the MP Biomedical Corticosterone RIA kit for rats and mice (Orangeburg, NY) with the following changes: the hamster serum samples were assayed at a 1:50 dilution, and the primary antibody was diluted 1:2 in sample diluent. The limit of detection was 6.25 ng/ml. All samples were assayed using 2 kits for which the interassay CV was 11.7% for a kit control sample consisting of corticosterone in assay diluent that inhibited binding to 23%.

Statistics

LH surges, which consist of at least 4-fold increases in LH levels that last ~4-5 hours in an individual animal, were defined as at least 2 consecutive values greater than the 95% confidence limits of the group mean basal LH levels (at ZT 5), a criterion reported previously (Swann and Turek, 1985) (Duncan et al., 2014; Blake et al., 1983; Tsai and Legan, 2001). Once an LH surge is identified, the LH “peak” is the single maximum LH value for a given surge. The time of each LH peak was used as an objective phase reference point for the time of the LH surge. This method of identifying the peak has been reported to correspond well to the maximum value of a sine wave fitted to the data by a least squares technique (Moline et al., 1986). The group mean LH surge peaks and times of peak LH for Expts. 1 and 2 were compared between days or groups by Student's t-tests or by Mann-Whitney Rank Sum tests when the values were not normally distributed, as determined by the Shapiro-Wilk test, or had unequal variances as determined by Levene's test. LH peaks and times of peaks and mean plasma LH levels were compared for main effects of group and day by 2-way repeated measures ANOVA. Comparison of the time of the Day 2 LH peak among the home cage controls in Expt. 1 and the ovx+EB and intact hamsters in Expt. 2 was performed using a Kruskal-Wallis ANOVA on Ranks. In cases of significant interactions of main effects, Holm-Sidak post hoc tests were performed. Circulating corticosterone levels (Expt. 4) and wheel revolutions during the initial six hours of novel wheel exposure (Expt. 2) were analyzed by repeated measures ANOVA and Holm Sidak post hoc tests, as above. Wheel revolutions were analyzed by 2-way repeated measures ANOVA. Phase shift values obtained from Clocklab in Expt. 2 were analyzed by 1-way ANOVA and t test. All above statistical analyses were performed using SigmaPlot (Systat Software Inc., San Jose, CA). Phase shift values in Expt. 3 were analyzed with Prism (Graphpad Software, San Diego, CA) by unpaired t-test with Welsch's correction for unequal variances. Data are reported as mean $\pm$ SE and the significance level was $P < 0.05$. Effect size was calculated as eta squared (η^2) for ANOVAs and Cohen's d for pair-wise comparisons only when the data were distributed normally and had equal variances.

Results

Experiment 1: Daily LH surges in ovx+EB hamsters

As expected, EB injection elicited two consecutive daily LH surges in ovx hamsters that were transferred to a new room and exposed to DD at ZT 5 (Fig. 1A). The Day 2 LH peak was 46% as high as the Day 1 LH peak (18.8 ± 4.0 vs 40.9 ± 7.9 ng/ml, $P = 0.038$), and occurred about 1 hour earlier (LH peak at $ZT 7.0 \pm 0.1$ h vs $ZT 8.1 \pm 0.2$, $P = 0.002$). The decrease in amplitude of the Day 2 LH surge is commonly observed for repeating daily LH

surges (Legan and Karsch, 1975; Legan et al., 1975; Stetson et al., 1978), and is most likely due to the depletion of pituitary LH content following each daily LH surge. The timing of the peak of the Day 1 LH surge (ZT 8.1) suggests that EB treatment, transfer to a new room and exposure to DD advanced the LH surge by about 1 h, because previous findings of our own and others show that in intact proestrous hamsters, LH surges peak at about ZT 9 (Stetson et al., 1978; Duncan et al., 2014). In contrast, the phase of the circadian rhythm of locomotor activity was advanced by almost 2 hours (1.80 ± 0.35 h), as depicted in the representative actogram in Fig. 1B.

Experiment 2: Comparison of the effect of a novel wheel on daily LH surges and circadian phase shifts in ovx+oil, ovx+EB and intact proestrous hamsters

Day 1 LH surges—Exposure of intact proestrous hamsters to a novel wheel at ZT 5 blocked the Day 1 LH surge (Fig. 2A-C, bottom panels), as we have reported previously (Legan et al., 2010; Duncan et al., 2014). In the wheel-exposed ovx+EB hamsters, presentation of a novel wheel did not completely abolish the Day 1 LH surge, but greatly attenuated it, with the peak averaging 8.8 ng/ml, or about 22% of that in control ovx+EB hamsters (compare middle panels of Fig. 2A with Fig. 1A). In oil-treated ovx hamsters, plasma LH levels on Day 1 were higher than those in the intact and ovx+EB groups (Fig. 2A, top, main effect of group, $P < 0.001$, $F_{2,6} = 61.985$). These elevated basal LH levels are characteristic of those observed in ovx hamsters housed in long days in the absence of estradiol and wheel exposure (Goldman et al., 1971; Jorgenson and Schwartz, 1984). An elevation in plasma LH concentrations met the criteria for an LH surge in only one ovx+oil treated hamster on Day 1, when a peak level of 31.76 ng/ml was attained at ZT 7.

Day 2 LH surges—In the intact proestrous hamsters, an LH surge occurred on Day 2 that peaked on the average at ZT 7, two hours earlier than normally occurs in non-wheel exposed proestrous hamsters, as we reported previously (Legan et al. 2010). OvX+EB hamsters also exhibited a robust LH surge that peaked 1.7 hours earlier than the Day 1 LH surge (time of Day 2 vs Day 1 peak: $ZT 6.5 \pm 0.2$ vs $ZT 8.2 \pm 0.3$, Fig 2A-C, middle panel, $P = 0.001$, $t = 4.385$, $df = 10$). Thus the timing of the peak of the Day 2 LH surge in wheel-exposed ovx +EB hamsters (ZT 6.5) was similar to that in intact hamsters (ZT 7.0) and to that of the Day 2 LH surge in home-cage control hamsters (ZT 7.0) (Compare Fig. 2C, middle and bottom panels and Fig. 1, top right panel, $P = 0.193$). The dramatic increase in peak levels of the Day 2 LH surges in the ovx+EB and intact hamsters (Fig. 2B, middle and bottom panels) is most likely due to accumulation of pituitary LH following blockade or suppression of the LH surges on Day 1, as has been observed following barbiturate blockade of LH surges (Legan et al., 1975; Stetson et al., 1981). There were no LH surges on Day 2 in the ovx+oil-treated group.

Locomotor activity—Wheel revolutions per hour on Day 1 did not differ among the groups (Fig. 3, $P = 0.054$, $F_{2,5} = 3.445$). Novel wheel exposure phase advanced the locomotor activity rhythm by about 1.5 to 3 h in all 3 groups, but treatment effects were not significantly different (Fig. 4A, $P = 0.082$, $F_2 = 2.915$, $\eta^2 = 0.2554$). However, when the intact and ovx+E groups were combined in order to determine the effect of estradiol on the novel wheel-induced phase advances, the phase advance in this combined group was about 2-fold

greater than that in the oil-treated ovx hamsters (Fig. 4B, $P=0.029$, $t=2.368$, $df=18$, $d=1.1555$).

Experiment 3: Can arousing stimuli other than a novel wheel block and phase advance the LH surge and the circadian activity rhythm?

When ovx+EB hamsters were exposed to a novelty paradigm (sequential exposure to toys) from ZT 4-6, the Day 1 LH surge was not blocked but was attenuated (Fig. 5A bottom, main effect of treatment, $P=0.037$, $F_{1,1}=5.193$), resulting in an average peak LH level that was 60% of that in home cage controls. Toy exposure also delayed the time of the Day 1 LH peak by an average of 0.9 h (Fig. 5C bottom, group x time interaction, $P=0.001$, $F_{1,1}=16.493$). Day 2 LH surges were higher in amplitude than the Day 1 LH surges in both toy-exposed and control hamsters (Fig. 5A & B, bottom, main effect of Day, $P=0.027$, $F_{1,1}=6.007$). Just as on Day 1, the Day 2 LH surges in toy-exposed hamsters were attenuated (Fig. 5A & B bottom, group x time interaction, $P=0.025$, $F_{1,1}=2.544$), but were not delayed, as the LH surges peaked on the average at ZT 6.8 in both groups (Fig. 5C bottom). As a result, in home cage controls Day 2 LH surges peaked an average of 0.9 h earlier than Day 1 LH surges, whereas in toy-exposed hamsters, the Day 2 LH surges occurred 2 h earlier than the delayed Day 1 surges. Thus toy exposure induced a 2-fold greater phase advance than transfer to a new room and exposure to DD alone.

Interestingly, toy exposure beginning at ZT 5 did not attenuate the Day 1 LH surge (Fig. 5A and B, top, main effect of group, $P=0.244$, $F_{1,6}=1.443$), or the Day 2 LH surge (main effect of group, $P=0.491$, $F_{1,1}=0.494$, $\eta^2=0.0142$). However, all remaining effects on the amplitude and timing of LH surges were the same as those following arousal begun at ZT 4. Namely, toy exposure beginning at ZT 5 delayed the Day 1 LH surge about 1 h (Fig. 5C, top, main effect of group, $P=0.014$, $F_{1,1}=7.406$, $\eta^2=0.0941$). In addition, the Day 2 LH peaks were greater than those on Day 1 (Figs. 5A and B, top, main effect of Day, $P=0.002$, $F=13.083$, $\eta^2=0.0244$). With respect to timing of the LH peak, on Day 2 the LH surge peaked at about ZT 6.7 in both groups, resulting in a 1 h phase advance in the home cage group and a 2 h advance in the toy-exposed animals as compared to the time of the LH peak on Day 1 (Fig. 5C, top). The locomotor activity rhythm was also phase advanced ~ 2 hours when the arousal paradigm was initiated at ZT 4 (Fig. 6A and actograms, $P=0.005$, $t=3.361$, $df=13$) but not when it was started at ZT 5 (Fig. 6B, $P=0.352$, $t=0.961$, $df=15$).

Surprisingly, insertion of a screen dividing the cage with another hamster on the other side of the screen did not lead to interactions between the two animals at the screen. Instead, most of the experimental hamsters investigated the screen intensely during the 30 minute period, including attempting to burrow under it, climbing on it, and pulling and biting on its edges. Similar behaviors were observed when the screen was later re-inserted and a different hamster was placed on the other side of it, except that investigation of the screen was more intermittent and alternated with other behaviors, such as grooming. Whenever presented with a new object, the hamsters investigated it by sniffing, walking around it, pushing it, and climbing on it and going inside it when possible. This investigative behavior alternated with eating, grooming, moving around the cage, or sitting still. The animals were less active during the last half hour and became still and some closed their eyes.

Experiment 4: Is blockade of the LH surge associated with a greater increase in plasma corticosterone?

LH surges occurred in 6 of 12 home cage proestrous hamsters, the peak level averaging 15.34 ng/ml, and occurring on the average at ZT 9.5. Novel wheel exposure blocked the LH surge in 10 of 12 proestrous hamsters, and peak LH levels in the remaining 2 animals averaged 16.13 ng/ml at ZT 9.0. In the 7 ovx+EB hamsters, the LH surge was partially blocked by the novel wheel, the peak level averaging 5.41 ng/ml at ZT 9.1 (data not shown).

In the absence of a novel wheel, plasma corticosterone concentrations remained low throughout the sampling period regardless of whether or not there was an LH surge (Fig. 7, top, main effect of group, $P=0.828$, $F_{1,8}=0.0499$). A non-significant increment in corticosterone level, which was due to a high level in one animal that lasted about 30 minutes, caused the only difference in circulating levels between the 2 groups at ZT 5.25 (asterisk, Fig. 7, top, interaction, $P=0.023$, $F_{1,8}=2.399$). In contrast, the pattern of plasma corticosterone levels in proestrous hamsters in response to a novel wheel depended on whether the LH surge was blocked or not (Fig. 7, middle panel, group x time interaction, $P<0.001$, $F_{1,8}=4.069$). Prior to novel wheel exposure, mean corticosterone levels at ZT 5 were not different between the proestrous hamsters that surged and those that did not (post hoc pairwise comparison, $P=0.227$, $t=1.224$). However, novel wheel blockade of the LH surge was associated with a 2-hour increase in circulating corticosterone lasting until ZT 7, when levels were still about 2-fold greater than those in hamsters that exhibited an LH surge (Fig. 7, middle, post hoc comparison, $P=0.048$, $t=2.026$). In the 2 hamsters that had an LH surge, novel wheel exposure at ZT 5 had no effect on subsequent plasma corticosterone levels. Interestingly, in the ovx+EB hamsters, in which novel wheel exposure partially blocked the LH surge, a modest elevation (main effect of time, $P<0.001$, $F_8=5.675$) in plasma corticosterone levels above those at ZT 5 occurred; however this rise only lasted for about an hour (Fig. 7, bottom). Thus, only those elevations in circulating corticosterone levels that last more than 1 h are associated with novel wheel-induced blockade of the LH surge.

Discussion

The foregoing experiments tested the hypotheses that: (1) arousal without a novel wheel has the same effect as a novel wheel in acutely blocking and subsequently phase advancing the LH surge; and (2) that circulating corticosterone levels are higher in those animals which respond to novel wheel exposure with LH surge blockade than in those that exhibit an LH surge. In order to test these hypotheses, the first experiment confirmed an experimental model characterized by daily LH surges for studying the effect of novel wheel exposure on LH surges. The results of Experiment 2 demonstrated that in ovx+EB hamsters, which exhibit daily LH surges, exposure to a novel wheel at ZT 5 has a similar, although somewhat milder effect on LH surges as in intact proestrous hamsters. Thus in ovx+EB hamsters, the LH surge on Day 1 is greatly attenuated rather than blocked; however just as occurs in intact hamsters, the LH surge on the next day is phase advanced by about 2 hours. Therefore the ovx+EB model is appropriate for investigating the effects of arousal on the circadian rhythm of LH secretion in females.

The deep suppression of the Day 1 LH surge in ovx+EB treated hamsters in Experiment 2, instead of the complete blockade that occurs in most intact proestrous hamsters, suggests that either ovariectomy and/or the positive feedback signal elicited by exogenous estradiol treatment strengthens the neural signal for the LH surge. In support of this possibility, ovx hamsters exposed to a long day photoperiod (14 h light/day) can exhibit daily afternoon increases in circulating LH levels in the absence of ovarian steroids (Shander and Goldman, 1978; Stetson et al., 1978; Stetson et al., 1981; Hauser and Benson, 1986; Jorgenson and Schwartz, 1984), although such afternoon increases in circulating LH levels do not always occur (Norman et al., 1973; Goldman et al., 1971; Seegal and Goldman, 1975; Jorgenson and Schwartz, 1984), as was observed in our ovx+oil treated hamsters. Further, estradiol enhances the daily afternoon increases in LH for the first few days after treatment (Norman et al., 1973; Stetson et al., 1978), even when daily LH surges do not occur prior to estradiol treatment (Jorgenson and Schwartz, 1984). Alternatively, the positive feedback signal generated by injection of 50 µg of exogenous EB in our study was likely stronger than the endogenous physiologic signal in intact proestrous hamsters since as little as 5 µg EB can elicit a proestrous-like LH surge in ovx hamsters (Norman and Spies, 1974). Further, administration of 50 µg EB in ovariectomized rats elicits serum estradiol levels that are about 40-fold greater at 10 hours post-injection and 4 days later are still 2-fold higher than those on the morning of proestrus (Legan and Karsch, 1975). With regard to the effect of removal of estradiol feedback during the 2 weeks after ovariectomy, the hypothalamic-pituitary axis would be maximally secreting GnRH and LH, and therefore its response to any suppressive stimuli for either the negative or positive modes of LH release would be decreased.

The differences between the amplitudes of the Day 1 and 2 LH surges in Expts. 1 and 2 are most likely due to changes in pituitary content of LH. In Expt. 1, the amplitude of the second daily LH surge is decreased because of a depletion of the reserve of pituitary LH after the LH surge on the preceding day. In contrast, in Expt. 2, the second daily LH surge is greater because the Day 1 LH surge was either blocked or attenuated, resulting in a larger releasable pool of pituitary LH, which is also further increased due to an additional day of exposure to the positive feedback action of the elevated circulating estradiol concentrations.

Experiment 3 investigated the effect of a sequential presentation of toys alone or with conspecifics to determine if suppression of the LH surge is unique to presentation of a novel wheel or could also be induced by a different arousal paradigm. In contrast to the effect of a novel wheel, the sequence of toys did not completely block the LH surge, but when presented at the appropriate time of day (beginning at ZT 4), suppressed the amplitude and delayed the LH surge on Day 1, and phase advanced the surge on Day 2 by about 2 h. Thus different arousing stimuli have different effects on the timing and amplitude of the LH surge. These differences may be due to the duration of the stimulus, i.e., continuous exposure to the novel wheel versus two hours of exposure to various toys. Alternatively, the differential effect of a novel wheel versus toys may be due to differences in strength of the arousal stimulus. Wakefulness stimulated by novel wheel presentation is maintained for at least 5 hours based on the wheel revolutions, whereas wakefulness induced by toys diminishes after approximately 90 minutes, based on our continuous observations. Therefore

the different effects of a novel wheel versus toys on the LH surge may be related at least in part to their effectiveness in maintaining wakefulness and/or physical activity.

Our findings that transport to a new room does not affect the LH surge, whereas toy exposure greatly suppresses the LH surge, and novel wheel exposure completely blocks the LH surge in most hamsters suggest that a threshold level of arousal is required to suppress the LH surge and that activity potentiates arousal. In addition the effects of toy exposure on timing of LH surges in ovx+EB hamsters are different from those in intact proestrous hamsters exposed to a novel wheel. Following a 2-h arousal paradigm, the Day 1 LH peak is delayed 1 h, whereas after novel wheel exposure, if the Day 1 LH surge is not blocked, its phase is not altered (Duncan et al., 2014). In toy-exposed hamsters, the Day 2 surge peaks 2 h earlier than the Day 1 surge; however it is actually phase advanced only 1 h relative to that in the home cage controls. In novel wheel exposed hamsters whose LH surge was blocked on Day 1, the LH surge on Day 2 is phase advanced 2 h. These results suggest that activity potentiates the phase-advancing effect of arousal.

Another cause for the different effects of novel wheel exposure versus arousal on amplitude and timing of LH surges may result from phase shifts in the neural signal for the LH surge. Further, the greater effectiveness of the arousal paradigm in both suppressing the LH surge and in phase advancing the circadian rhythm of locomotor activity rhythm when initiated at ZT 4 rather than at ZT 5 indicates that the timing of the arousal stimulus is critical and supports the hypothesis that arousal may interfere with the daily neural signal for the LH surge. Based on the time when barbiturates can block the LH surge in intact proestrous hamsters, the critical period when this circadian timing signal triggers the LH surge begins between ZT 5 and ZT 6, and lasts about 2 hours, with the LH surge peaking at ZT 9 (Goldman and Mahesh, 1969; Greenwald, 1971; Norman et al., 1973; Legan et al., 2009). Thus, if a nonphotic stimulus only spans a portion of the neural signal's timespan because it is being phase advanced, then the LH surge might still occur, but would be attenuated and delayed. The greater the proportion of the neural signal that is blocked, i.e., the greater the phase advance in the neural signal in response to a nonphotic stimulus, the smaller the amplitude and the greater the delay in the LH surge. Complete blockade of the neural signal would abolish the LH surge for the next ~22 h, in the case of a 2 h phase advance. Since the LH surge in ovx+EB hamsters peaks an hour earlier (ZT 8, Fig. 1), it is likely that the critical period in these hamsters begins at ZT 4. Thus, in ovx+EB hamsters, the arousal stimulus may only completely overlap with the critical period for the LH surge when the stimulus begins at ZT 4, but not at ZT 5. Consequently, initiation of the arousal paradigm at ZT4 resulted in a suppressed, delayed LH surge, whereas initiation of the arousal paradigm at ZT 5 resulted in a delayed, but not attenuated, LH surge. The unexpected increase in amplitude of the Day 2 LH surges compared to those on Day 1 in all groups may also reflect the proportion of the neural signal given on each day. Nonphotic stimuli cause very rapid phase shifts; thus on Day 1, if the nonphotic stimulus at ZT 4-5 initiates a phase advance in the neural signal, there might only be a partial signal by ZT 6-7 when the LH surge begins. However by Day 2, the phase shift would be complete resulting in a full neural signal and a larger, phase advanced LH surge.

Another possible factor contributing to the effectiveness of a non-photic signal in suppressing the LH surge may be the resultant increase in circulating glucocorticoid levels. Although it is well established that chronic stress or elevation of plasma glucocorticoids decreases basal circulating LH levels and is capable of blocking the LH surge in rats (Rivier and Rivest, 1991; Roozendaal et al., 1995; Baldwin, 1979; Kam et al., 2000), the effects of acute, mild stress on LH concentrations are variable and are dependent on the type and intensity of the stress and on age and gender (Briski, 1996). Even a mild acute stress can suppress the LH surge. For example in rats, restraint stress for one hour (ZT 4-5) on the morning of proestrus suppresses the afternoon LH surge (beginning at ZT 10) by about 75% (Donadio et al., 2007). The restraint stress induced a transient increase in plasma corticosterone that lasted ~2-3 h. Thus, if novel wheel exposure increases corticosterone levels for a few hours in the late morning, then this transient elevation could constitute part of the mechanism that leads to suppression of the late afternoon LH surge. In this regard, a number of previous studies have demonstrated that transfer of male rats to a new cage and/or exposure to a novel wheel induces a transient increase in circulating glucocorticoid levels (Reul et al., 1993; Seggie and Brown, 1975; Baldwin et al., 1974; Buijs et al., 1997). Whether this also occurs in females has not been reported. Furthermore, the stress axis exhibits fundamental differences between rats and hamsters. In female rats, both basal and stress-induced levels of circulating glucocorticoids are higher than those in males (Viau et al., 2005; Atkinson and Waddell, 1997), whereas in hamsters, glucocorticoid levels are higher in males than in females (Gaskin and Kitay, 1970; Weinberg and Wong, 1986). In addition, hamsters secrete both cortisol and corticosterone (Albers et al., 1985), and have much lower levels of corticosterone than rats, in whom corticosterone is the predominant circulating glucocorticoid.

Therefore, Experiment 4 tested the hypothesis that in those hamsters whose LH surge was blocked by novel wheel exposure, the wheel induced a greater increment in circulating corticosterone levels than in those hamsters whose LH surge was not blocked. Our results support this hypothesis and demonstrate for the first time that novel wheel blockade of the LH surge in hamsters is associated with an increase in circulating corticosterone levels that is sustained for approximately 2 hours and thus can be considered a mild stress. It remains to be determined whether toy exposure also causes increases in plasma corticosterone levels in association with suppression of LH surges in hamsters, and whether they are similar to those that occur after introduction of a novel wheel. In this regard, it is important to note that stress is also accompanied by increases in numerous other hormones that can interfere with LH surges and may play a role in the suppressive effects of non-photic stimuli on LH surges, e.g. CRH (Traslavina and Franci, 2011), POMC-related peptides (Rivier and Rivest, 1991), biogenic amines (Rivier and Rivest, 1991), and progesterone (Donadio et al., 2007; Traslavina and Franci, 2011; Hueston and Deak, 2014), to name a few.

The results of Experiment 4 are also the first to demonstrate that acute, transient, 2-fold increases in corticosterone levels occur in female hamsters during exposure to a novel wheel in association with phase shifts of the master circadian pacemaker. The possible role of glucocorticoids in mediating nonphotic phase shifts in activity rhythms has been investigated in male hamsters. Mistlberger et al. reported that mildly stressful confinement to a wheel, which causes large phase advances in circadian locomotor activity, does not

significantly increase serum total cortisol levels at 30, 90 or 180 minutes after confinement (Mistlberger et al., 2003). In contrast, in the same study, physical restraint combined with continuous blasts of air is highly stressful, causing a 5-fold increase in cortisol after 90 and 180 minutes, without inducing significant phase shifts. Exogenous infusion of cortisol (0.1 mg/24 h s.c. administered continuously, or in a 6-h pulse beginning at ZT 12 or ZT 3), or injection of dexamethasone (1 or 5 mg i.p.) at ZT 4 or 6 does not alter the phase of locomotor activity rhythms in male hamsters (Albers et al., 1985; Janik et al., 2001). These reports suggest that elevated glucocorticoid levels do not mediate the phase shift in circadian activity rhythms induced in male hamsters by novel wheel confinement or sleep deprivation. However, it is important to note that Mistlberger et al. did not obtain frequent serial samples within the first 1-2 hours of any arousal stimulus known to cause phase shifts (Mistlberger et al., 2003). Therefore this study would have missed an early, transient rise in circulating glucocorticoid levels, such as we observed in Experiment 4. Further, the replacement studies used a supraphysiologic dose of glucocorticoid or a long-lasting synthetic glucocorticoid with limited ability to cross the blood brain barrier (Albers et al., 1985; Janik et al., 2001). Therefore, it remains to be determined whether a transient, physiological increase in serum corticosterone levels during the first 2 hours or so of a non-photic stimulus mediates its circadian phase resetting effect. In hamsters, the relative role of corticosterone versus cortisol or other hormones potentially released in response to nonphotic phase resetting stimuli also remains unknown in both males and females.

The foregoing results extend previous reports of non-photically-induced phase advances in circadian activity rhythms by demonstrating that sequential presentation of different toys also induces this effect in female hamsters. This finding is similar to previous reports that a variety of non-photic stimuli that are associated with arousal, such as forced wheel running, exposure to estrous odors, administration of Triazolam, or subcutaneous saline injection, induce phase advances in activity rhythms in male hamsters (Mrosovsky and Salmon, 1990; Turek and Van, 1989; Hastings et al., 1992).

What are the neural mechanisms by which various non-photic stimuli induce circadian phase shifts? There are a plethora of inputs to the GnRH neurons that influence the LH surge, e.g., kisspeptin, GABA, glutamate, serotonin, dopamine, norepinephrine, orexin, and NPY, to name a few, and changes in any or all of these inputs might participate in blocking the LH surge. Thus, in male hamsters, NPY induces phase advances when injected into the SCN in the midsubjective day (Huhman and Albers, 1994). Further evidence for a major role for NPY showed that in male hamsters novel wheel exposure and other nonphotic zeitgebers activate neurons in the thalamic intergeniculate leaflet (IGL) that release neuropeptide Y (NPY) and other neurotransmitters in the SCN (Mikkelsen et al., 1998; Muscat and Morin, 2006; Glass et al., 2010). In contrast to male hamsters, presentation of a novel wheel to female hamsters at ZT 5 induces Fos expression in orexin neurons that are involved in arousal and wakefulness, but not in neurons in the IGL (Duncan et al., 2014). The apparent differences between the effects of a novel wheel on IGL Fos expression in males versus females may be related to either the sex difference and/or the relatively small sample size and possible insufficient statistical power in the female hamster study. In both sexes, wakefulness without intense exercise is sufficient to induce phase shifts and to stimulate Fos expression in orexin-immunoreactive neurons (Antle and Mistlberger, 2000; Webb et al.,

2008; Duncan et al., 2014). However, the finding in male hamsters that some wake-inducing stimuli that do not induce phase shifts also stimulate Fos expression in orexin neurons suggests that activation of these neurons is not sufficient for nonphotic circadian phase shifts (Webb et al., 2008). In female hamsters, administration of an orexin-1 receptor antagonist reduces the magnitude of circadian phase shifts and prevents novel wheel blockade of the LH surge (Duncan et al., 2014). Furthermore, a locked wheel blocks the LH surge when other wake-promoting stimuli are also present, suggesting that wakefulness rather than exercise per se is necessary for this effect (Duncan et al., 2014). The present results extend these findings to ovx+EB hamsters and demonstrate for the first time that an arousal paradigm consisting of exposure to novelty without intense exercise from ZT 4-6 phase advances both the circadian activity rhythm and differentially modifies the timing and amplitude of Day 1 and Day 2 LH surges.

The results presented herein expand our previous finding that the intense exercise associated with exposure to a running wheel is not absolutely required for suppression of the LH surge or phase shifting the master pacemaker in intact proestrous hamsters. Although the circadian timing signal for the LH surge may be stronger after ovariectomy, and/or the positive feedback effect of the exogenous EB injection is greater than that of the endogenous preovulatory increment in circulating estradiol levels, the basic phenomenon of arousal-related phase resetting and suppression of the LH surge is similar in intact proestrous and estradiol-treated ovariectomized hamsters.

In conclusion, these studies sought to further elucidate the mechanisms by which a novel wheel blocks the LH surge and induces circadian phase shifts by testing the effect of a different arousal paradigm and delineating the pattern of circulating corticosterone levels after novel wheel exposure. The results indicated that an arousal paradigm (i.e., sequential presentation of novel toys for 2 hours) could acutely decrease the LH surge on Day 1, but was not as effective as a novel wheel, which induced more prolonged wakefulness and activity. The endocrine effects of novel wheel exposure include changes in circulating corticosterone levels as well as changes in LH. Circulating corticosterone levels increased within 15 minutes of novel wheel exposure and remained elevated for ~2 hours in hamsters in which the Day 1 LH surge was blocked, but not in those in which the LH surge occurred. This finding suggests activation of the stress axis may participate in mediating blockade of the LH surge by acute arousal. These arousal-induced changes in circadian rhythms and circulating hormone levels may be relevant to human health, because shift work, jet travel, and on-call schedules involve acute increases in wakefulness during previous rest periods. Interestingly, in animal models of shiftwork the phase relationships of a number of overt circadian rhythms throughout the body are disrupted (Davidson et al., 2009; Castanon-Cervantes et al., 2010; Salgado-Delgado et al., 2013; Wolff et al., 2013). In humans, similar disruptions among clock gene expression rhythms and circadian rhythms in circulating hormone levels also occur, and female shift workers have increased risks of irregular menstrual cycles, miscarriages, infertility, and other reproductive problems (Gamble et al., 2013).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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HIGHLIGHTS

- Novel wheel suppresses LH surges better in intact than estrogen-treated ovariectomized hamsters
- Toy arousal at ZT4, but not ZT5, suppresses the LH surge and resets the phase of the master pacemaker
- Arousal with toys is not as effective as a novel wheel in decreasing LH surges
- Plasma corticosterone increases transiently immediately after novel wheel exposure
- Novel wheel exposure only blocks LH surges when the corticosterone increase is prolonged ~ 2 hours

Fig. 1.

Daily LH surges in ovx+EB hamsters transported to a new room and exposed to constant darkness (DD) at ZT 5 (vertical arrow). (A) Mean $\pm$ SE plasma LH concentrations from serial samples obtained from ZT 3-10 on Days 1 and 2 after administration of EB s.c. (B) Record of wheel running activity (black marks) of a representative ovx+EB hamster. The x axis represents time of day (hours). ZT is indicated by the numbers across the top of the actogram. The y axis represents sequential days. When the hamster was exposed to a 14L:10D photoperiod (cross-hatched vertical bar), activity began at approximately lights off (ZT 12). After 2 weeks, the hamster was moved to a new room at ZT 5 (asterisk) and exposed to DD (solid vertical bar); this combined treatment induced a 2.24 h phase advance in the activity rhythm. The period length (τ) in DD for this hamster was 23.84 h. The τ in DD for all animals was 23.80 ± 0.03 h.

Fig. 2.

Effect of a novel wheel on mean $\pm$ SE plasma LH concentrations. (A) Plasma LH concentrations in ovx+oil treated hamsters (upper panel) were unchanged after exposure to a novel wheel and DD (arrow). However, novel wheel exposure greatly dampened LH surges on Day 1 in EB-treated ovx hamsters (middle panel), and completely blocked Day 1 LH surges in intact proestrous hamsters (lower panel). The inset in the middle panel provides a magnified graph of the LH surge in novel wheel-exposed ovx+EB hamsters calibrated on an altered vertical scale with higher resolution. (B) Similar high amplitude LH surges occurred on Day 2 in ovx+EB hamsters (middle panel) and intact proestrous hamsters (lower panel) after novel wheel exposure had either greatly suppressed or blocked the Day 1 LH surges. Mean $\pm$ SE peak LH levels differed between Days 1 and 2 in ovx+EB hamsters (middle panel) but not in ovx+oil animals (upper panel). (C) In ovx+oil hamsters (upper panel), novel wheel exposure did not affect the LH peak times, which did not differ between Day 1 and Day 2. In ovx+EB hamsters (middle panel), the LH peak occurred 1.7 h earlier on Day 2

than on Day 1 (ZT 6.5 vs 8.2), and these times were similar to those in home cage controls (ZT 7.0 vs 8.1, Fig. 1). In intact hamsters (lower panel), the Day 2 LH peak occurred on the average at ZT 7.0 similar to the peak time in the ovx+EB group.

Fig. 3.

Effects of ovariectomy and EB on acute wheel running after exposure to a novel wheel (vertical arrow) and DD (shaded area). Mean $\pm$ SE cumulative wheel revolutions are depicted by the closed circles and solid lines. The open circles indicate the values for individual hamsters.

Fig. 4.

(A) Novel wheel-induced phase advances in circadian activity rhythms are similar in intact, ovx+oil, and ovx+EB hamsters. (B) The average phase advance in intact proestrous and ovx +EB hamsters combined, conditions characterized by elevated circulating estradiol levels, was greater than that in oil-treated ovx hamsters. The tau in DD for the ovx+oil group was 23.78 ± 0.21 .

Fig. 5.

Effect of exposure to a 2-hour arousal paradigm on LH surges in ovx+EB hamsters. (A) When presented from ZT 5-7, the arousal paradigm delayed the LH surge one hour without affecting its amplitude (upper left panel). Arousal from ZT 4-6 not only delayed, but also attenuated the LH surge (lower left panel). On Day 2 (right panels), the LH surges in all groups were phase-advanced and increased in amplitude, compared to the Day 1 LH surges. (B) Mean (+SE) peak LH levels on Day 1 were suppressed by arousal from ZT 4-6 (lower left panel) but not from ZT 5-7 (upper left panel). Average peak LH levels did not differ between the treatment groups on Day 2 (right upper and lower panels). (C) The LH peak on Day 1 was delayed about 1 h following the arousal paradigm (left panels). On Day 2, the LH peak was phase advanced 1 h in home cage controls, and 2 h in toy-exposed hamsters (right panels) compared to the respective peak times on Day 1. The range of n for the Home Cage group (6A, upper panel) reflects loss of the last 3 samples in 2 hamsters, and of the last 2 samples in one hamster, which occurred at least 2 h after the peak in all 3 cases.

Fig. 6.

Exposure to a 2-h arousal paradigm (Toys) induced phase advances in locomotor activity rhythms when the paradigm was initiated at ZT 4 (A) but not at ZT 5 (B). Bars represent mean + SE; numbers in the bars indicate sample size (n). Actograms (bottom panels) depict phase shifts in a representative Home Cage hamster (left) and Toy-exposed hamster (right) transferred from a 14L:10D photoperiod (cross-hatched vertical bars) into DD (black vertical bar) at ZT 4. Numbers at the bottom of the actograms indicate ZT in hours.

Fig. 7.

Mean ($\pm$ SE) circulating corticosterone levels in home cage (upper panel) or novel wheel exposed hamsters (middle and lower panels) exhibiting an LH surge or not. The asterisks indicate values that are different between the groups as determined by two-way repeated measures ANOVAs. In the lower panel, the points designated as having $P < 0.004$ are different from the value at ZT 5. The very brief (~30 min) increase in mean plasma corticosterone levels in home cage proestrous hamsters (upper panel) had no effect on incidence of LH surges. In novel wheel-exposed (vertical arrow) proestrous (middle panel)

or ovx+EB hamsters (bottom panel), LH surges only occurred when the initial increase in corticosterone lasted an hour or less, whereas a prolonged (~2 h) increase in corticosterone levels was associated with blockade of the LH surge. The range of n in the middle panel indicates several missing samples in one of the 2 proestrous hamsters that surged.