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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-hour 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 h. 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.



## Introduction

Coordination of endogenously generated 24-hour (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 h 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 h, 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 h indicating

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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 non-photic 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 modanifil (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-photic 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-photic 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-photic 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 (Experiment 1) or an infrared motion detector (Experiments 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