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Estradiol Suppresses Recovery of REM Sleep Following Sleep Deprivation in Ovariectomized Female Rats

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Abstract

Sleep complaints such as insufficient sleep and insomnia are twice as prevalent in women. Symptoms of sleep disruption are often coincident with changes in the gonadal hormone profile across a women's lifespan. Data from a number of different species, including humans, non-human primates and rodents strongly implicate a role for gonadal hormones in the modulation of sleep. In female rats, increased levels of circulating estradiol increase wakefulness and reduce sleep in the dark phase. In this study, we asked whether this reduction in sleep is driven by estradiol-dependent reduction in sleep need during the dark phase by assessing sleep before and after sleep deprivation (SD). Ovariectomized rats implanted with EEG telemetry transmitters were given Silastic capsules containing either 17- β estradiol in sesame oil (E2) or sesame oil alone. After a 24-hour baseline, animals were sleep-deprived via gentle handling for the entire 12-hour light phase, and then allowed to recover. E2 treatment suppressed baseline REM sleep duration in the dark phase, but not NREM or Wake duration, within three days. While SD induced a compensatory increase in REM duration in both groups, this increase was smaller in E2-treated rats compared to oils, as measured in absolute duration as well as by relative increase over baseline. Thus, E2 suppressed REM sleep in the dark phase both before and after SD. E2 also suppressed NREM and increased waking in the early- to mid-dark phase on the day after SD. NREM delta power tracked NREM sleep before and after SD, with small hormone-dependent reductions in delta power in recovery, but not spontaneous sleep. These results demonstrate that E2 powerfully and specifically suppresses spontaneous and recovery REM sleep in the dark phase, and suggest that ovarian steroids may consolidate circadian sleep-wake rhythms.

Keywords

Hormone; paradoxical sleep; sex differences; insomnia; electroencephalogram; menopause

1. Introduction

Women are at increased risk for insomnia or insufficient sleep compared to men (1–3). Converging evidence suggests that these differences may be related to women's unique gonadal hormone milieu. Both subjective and objective sleep disturbances in women

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frequently coincide with dramatic changes in the ovarian hormone profile such as at adolescence, pregnancy or menopause (4–6). In perimenopausal women, self-reported sleep quality and polysomnographic measures are related to gonadal steroid and gonadotropin levels (7). Hormone-associated alterations in sleep are also seen across the menstrual cycle in younger women. Rapid-eye movement (REM) sleep decreases and stage 2 NREM sleep increases in the luteal phase of the menstrual cycle in healthy women (8) and in women with premenstrual dysphoric disorder (9,10). Women taking exogenous hormones for menopausal symptoms or as birth control show subjective and objective changes in their sleep (11,12). Ovarian hormones can thus substantially impact sleep for much of a woman's life; however, the biological bases for such effects are poorly understood.

Animal studies, predominantly conducted in laboratory rodents, have greatly informed our understanding of the neurobiology underlying sleep behavior (13). Rodents are also a well-vetted model system for studying hormone action in the brain. Yet, there is a paucity of studies addressing the mechanisms of ovarian hormone modulation of sleep behavior. It has long been known that estradiol (E2) increases physical activity in female rats (14). Cycling female rats show increased locomotor activity near ovulation (15,16), which is abolished by ovariectomy (OVX) (17) and restored by either systemic (18) or central (19) implants of E2. This periovulatory increase in arousal is matched by a decrease in REM and NREM sleep in rats (20–25) and mice (26). OVX abolishes this periovulatory decrease in sleep (27–30), and exogenous E2 treatment suppresses sleep, especially REM sleep, during the dark phase in OVX rats (27,28,31) and mice (30). It is thus clear that E2 powerfully influences spontaneous sleep in both intact and OVX/hormone-replaced animals.

While the cellular mechanisms underlying E2-mediated suppression of sleep are not well understood, our previous work along with others implicates a role for the ventrolateral preoptic area (VLPO), a clearly defined sleep-active nucleus (32,33). E2 decreases Fos-immunoreactivity in the VLPO (25,34) and decreases expression of lipocalin-prostaglandin-D2 synthase (25), which synthesizes the somnogen prostaglandin D2. Curiously, changes in prostaglandin D2 activity are tightly associated with NREM sleep (32) (but see (35)), suggesting that E2's potent effects on REM sleep have a more widespread or multimodal action on sleep-wake regulation.

Sleep deprivation (SD) paradigms are useful tools to further investigate E2's effects on REM sleep suppression. Sleep is regulated by a homeostatic drive that increases sleep propensity as a function of time spent awake, and a circadian drive that alternately promotes sleep and wakefulness across the day (36,37). Thus, experimentally restricting sleep induces a compensatory increase or 'rebound' that is proportional to the duration of the SD, as well as to the stage of sleep that was lost (eg. REM vs NREM) (38,39). However, this rebound may be attenuated when circadian promotion of wakefulness is elevated (40,41). At proestrus (when E2 levels are increasing), female rats suppress REM sleep during the light phase, while suppressing both REM and NREM sleep in the dark phase (25). Despite the significant reduction in REM sleep in the light, rebound occurs only on the light phase of the following day (i.e. estrus) (20,24). Based on this, we hypothesized that elevated circulating E2 inhibits recovery sleep at night, as it has been shown to do for spontaneous sleep.

Although E2 was recently reported to increase REM recovery sleep relative to baseline in rats subjected to 6 h SD, absolute REM duration in the recovery period did not differ in E2-treated rats compared to oils (42). It is thus possible that a longer sleep deprivation would impact recovery sleep more powerfully, making any hormone-mediated suppression (or facilitation) more obvious. Furthermore, in that study the 18 h recovery period used for direct comparisons of hormone treatments aggregated recovery sleep from both the light and the dark phase, making it difficult to determine whether hormone treatment exerted phase-

specific effects on recovery sleep. To our knowledge, no additional studies exist that compare the response to SD in female rats given exogenous E2, although in mice E2 was recently shown to attenuate NREM recovery (30). We therefore asked whether rats given exogenous physiological doses of E2 would attenuate the recovery from a 12 h SD covering the entire light phase. The SD and recovery schedule were designed to enhance the partial sleep suppression seen in the light phase of proestrous rats, while assessing recovery in the dark phase, when both exogenous and endogenous E2 suppress REM and NREM sleep. To determine whether recovery required additional time, we monitored sleep for an additional 24 h after the first recovery period. Additionally, sleep recordings were initiated within 72 h of hormone replacement; although not directly comparable to the proestrous increase in E2, this time scale allowed us to determine whether exogenous E2 could influence spontaneous sleep within a relatively rapid time frame.

2. Materials and Methods

2.1. Animals

Adult female Sprague-Dawley rats ($n = 10$; Charles River, Kingston, NY) were singly housed in polyethylene cages under a 12:12 light:dark cycle, with lights-on = Zeitgeber time (ZT) 0 and lights-off = ZT 12 (43). Animals received *ad libitum* access to food and water for the duration of the experiment. Animals weighed 275–300 g at the start of the study. All surgical and experimental procedures took place during the animals' light phase. All procedures were performed in accordance with the University of Maryland Institutional Animal Care and Use Committee and the NIH *Guide for the Care and Use of Laboratory Animals*.

2.2. Surgical and hormone treatments

Figure 1 outlines the chronology of the experimental design. Upon arrival, animals were ovariectomized and implanted with telemetry units for recording EEG and EMG activity under isoflurane anesthesia. A single 3–4 cm longitudinal incision was made through the skin on the dorsal abdominal surface, and single incisions were made in the muscle wall on each flank. Ovaries were extracted and clamped between the oviduct and uterus, then removed. The uterine horn was then replaced into the abdominal cavity and the muscle wall was sutured. A second 3 cm longitudinal skin incision exposed the skull and neck muscle. Using a handheld drill, two burr holes were drilled and stainless steel screw electrodes (Plastics One; Roanoke, VA) were implanted at +2.0 mm AP/ +1.5 mm LM and –7.0 mm AP/ –1.5 mm LM relative to bregma. The telemetry transmitter (TL11M2-F40-EET; Data Sciences International, St Paul, MN) was inserted subcutaneously into a blunt-dissected pocket to the right or left of the midline body, and electrode leads were threaded subcutaneously to the scalp incision. EEG leads were wrapped around the screw electrodes, screws were tightened flush with the skull and secured with dental cement. EMG leads were inserted directly into the neck muscle just left of the midline, approximately 1.5 mm apart. The scalp was sutured and the body incision was closed with wound clips. Animals were treated postoperatively with antibiotic ointment, topical lidocaine and 0.1 cc buprenorphine, and allowed to recover for at least 7 days prior to the start of experiments.

At the start of the experiment, animals were implanted subcutaneously with a 2 cm long Silastic capsule containing either 17- β estradiol (E2) (Sigma, St. Louis MO) in sesame oil (150 μ g/mL; $n = 5$), or sesame oil alone ($n = 5$) under isoflurane anesthesia shortly after lights-on. Following implantation, animals were returned to their home cage until the start of the baseline recording three days later. Delivery of E2 via Silastic capsules results in physiological serum concentrations within 24 hours of implantation and produces stable levels of serum E2 for at least 3–4 weeks (44–47). Based on this, serum E2 was sampled at

the conclusion of the study (eight days after implant) to assess circulating E2 concentrations during the study.

2.3. Sleep EEG data collection and analysis

EEG and EMG data were continuously collected using a PC running Dataquest ART 4.0 software (DSI). Home cages containing the implanted animals were placed on receiver bases that relayed transmitter data to the PC. Digitized signal data were scored offline with Neuroscore 2.0 (DSI). For each individual, a representative continuous block of the data record was hand-scored in 10-second epochs as wake (low-amplitude, high-frequency EEG combined with high-amplitude EMG), NREM (high-amplitude, low-frequency EEG and low-amplitude EMG) or REM (low-amplitude, high-frequency EEG with very low EMG tone). The hand-scored section was then used to calibrate the software's autoscoring algorithm to at least 95% agreement for each stage, and the calibrated autoscoring algorithm was applied to the entire record. Reliability was confirmed and corrected where necessary via visual inspection of the autoscored record.

2.4. Experimental schedule

EEG and EMG data was continuously collected throughout the length of the experiment (Fig. 1) with the third day after implant being set as the baseline. The first two days were removed from analysis to rule out possible residual effects of the capsule implant procedure and isoflurane anesthesia on sleep-wake state. On the fourth day, all animals were sleep-deprived (SD) via gentle handling (24) for 12 h starting at lights-on. This method used light auditory stimuli and introduction of novel objects to the cage to keep animals from sleeping, thereby minimizing potential confounds to the sleep deprivation introduced by forced locomotion. To further minimize stress to the animals during SD, animals were kept in their home cage for the duration of the deprivation. At the conclusion of the deprivation (lights-off), animals were recorded undisturbed for the remaining dark phase part of the day (recovery 1), and for the next 24 h (recovery 2; Fig. 1). Animals were sacrificed on the third day following SD. At the time of sacrifice, trunk blood was taken and processed for detection of serum E2 via radioimmunoassay as previously described (48). The RIAs were performed at the University of Virginia Center for Research in Reproduction Ligand Assay and Analysis Core (supported by the Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institute of Health, Specialized Cooperative Centers Program in Reproduction and Infertility Research Grant U54-HD28934).

2.5. Data Analysis and Statistics

Total sleep duration (min), total bout number and average bout duration were analyzed in 12 h bins for each vigilance state (REM, NREM, Wake) for the light and dark phase of the three experimental days (baseline, SD/recovery 1, and recovery 2). In addition, total REM, NREM and wake duration were analyzed separately in 4-h bins for each day. To control for baseline differences in sleep duration between hormone treatments (see Results), recovery REM and NREM sleep following SD were normalized to baseline REM and NREM sleep using a gain-loss ratio (42), where (gain-loss ratio = [recovery – baseline D phase] / [baseline L phase – SD]). NREM delta power was calculated in Neuroscore as a percentage of the EEG spectrum from 0 – 25 Hz.

Baseline vigilance state data (REM, NREM, wake duration) were analyzed two ways: 12 h totals for the light and dark phase were analyzed by a mixed-factor ANOVA comparing phase (light, dark) by hormone treatment (E2 × oil) in Statistica (Statsoft; Tulsa, OK). 4 h totals of vigilance state and NREM delta power data for the baseline, recovery 1 and recovery 2 days were analyzed by separate mixed-factor ANOVAS for the light and dark phases comparing ZT (light phase, 0, 4, 8; dark phase, 12, 16, 20) by hormone treatment (E2

× oil). Total bout number and average bout duration in the light and dark phases were analyzed by a repeated-measures ANOVA comparing day (baseline, SD/Recovery 1, Recovery 2) by hormone treatment (E2 × oil). Where appropriate, significant effects were followed up with Fisher LSD posthoc analysis. Gain-loss ratios were analyzed by a mixed-factor ANOVA comparing ZT (12, 16, 20) by hormone treatment (E2 vs oil). Serum E2 levels were analyzed by a student's *t*-test (E2 vs oil). All effects were considered significant at $p = 0.05$.

3. Results

3.1. Serum E2

Serum E2 levels were significantly elevated in E2-treated rats compared to the oil controls ($t_9 = 2.59$, $p = 0.027$; Fig. 2). At the time of collection (~3–4 days after SD and recovery), the E2 levels were within a physiological range equivalent to the diestrus day when E2 are rising, but have not yet reached their peak (49,50).

3.2. Baseline sleep

12-hour totals of REM, NREM and wake revealed robust daily rhythms in all animals (REM, $F_{(1,8)} = 73.661$, $p < 0.001$; NREM, $F_{(1,8)} = 160.590$, $p < 0.001$; wake, $F_{(1,8)} = 195.271$, $p < 0.001$; Fig 3A, C, E). When the light and dark phases were analyzed independently, E2 significantly reduced total dark phase REM by 60% compared to the oil treated control ($t_9 = 3.304$, $p = 0.009$). Further analysis of 4 hr epochs across the light and dark phases revealed that this difference in spontaneous REM sleep was most prominent over the first 8 hours of the dark phase although only the main effect of hormone treatment reached significance (hormone, $F_{(1,8)} = 13.119$, $p = 0.007$; hormone × ZT, $F_{(2,16)} = 2.719$, $p = 0.096$; Fig. 3B). By contrast, in the light phase, REM sleep duration was unaffected by E2 (hormone, $F_{1,8} = 0.154$, n.s.; hormone × ZT, $F_{2,16} = 0.875$, n.s.).

E2 treatment did not influence 12-hour totals for baseline NREM or wake in either phase. There were no time-dependent effects of E2 on baseline NREM sleep in the light phase (hormone, $F_{1,8} = 0.214$, n.s.; hormone × ZT, $F_{2,16} = 0.339$, n.s) or the dark phase (hormone, $F_{1,8} = 0.067$, n.s.; hormone × ZT, $F_{2,16} = 1.248$, n.s; Fig. 3D). Baseline wake duration was likewise unaffected by E2 in the light phase (hormone, $F_{1,8} = 0.627$, n.s.; hormone × ZT, $F_{2,16} = 0.106$, n.s) or the dark phase (hormone, $F_{1,8} = 0.534$, n.s.; hormone × ZT, $F_{2,16} = 1.786$, n.s; Fig. 3F). E2 thus suppressed spontaneous REM sleep in the early- to mid-dark phase within 72 hours of capsule implantation, while leaving spontaneous NREM sleep and wake unaffected over the same time span.

3.3. SD and recovery

3.3.1. SD—SD increased waking to a similar extent in E2- and oil-treated rats (E2, 316% of baseline, oil, 345 % of baseline; $t_9 = .119$, n.s.). SD eliminated ~95% of baseline REM sleep and ~80% of NREM sleep over the 12 h light phase in both E2 and oil-treated rats (Fig. 4A, B). Further analysis of residual NREM sleep during the SD period revealed that when NREM bouts occurred these episodes were short (average episode duration, E2 = 38.5 s; oil = 42.8 s), and that neither bout duration nor bout number differed between E2- and oil-treated rats (total NREM bout number, $t_9 = 0.059$, n.s.; average NREM bout duration, $t_9 = 0.248$, n.s.).

3.3.2. REM sleep—Upon release from SD, both E2- and oil-treated rats exhibited elevations in REM duration over baseline values; however, E2 attenuated REM duration compared to oil at ZT 12 and ZT 16 (ZT × hormone, $F_{2,16} = 13.835$, $p < 0.001$; Fig. 4A). This attenuation persisted when total REM duration was normalized as a gain-loss ratio to

account for the effect of E2 treatment on baseline REM sleep (Fig 5A, B). E2 attenuated the relative gain in REM sleep over baseline at ZT 12 and ZT 16 compared to oil (ZT $\times$ hormone, $F_{2,16} = 6.767$, $p = 0.007$; Fig. 5A). When summed across the 12 h dark phase, the gain-loss ratios further revealed that E2-treated rats accumulated less recovery sleep compared to oil controls ($t_9 = 2.884$, $p = 0.018$; Fig. 5B). On recovery day 1, E2 thus suppressed both absolute REM duration and relative increases over baseline in the dark phase, compared to oil.

3.3.3. NREM sleep—Upon release from SD, E2-treated rats spent less time in NREM sleep than oil-treated rats at ZT 16 (ZT $\times$ hormone, $F_{2,16} = 15.196$, $p < 0.001$ Fig 4B). However, this difference was not reflected in a relative increase in NREM sleep duration compared to baseline (hormone, $F_{1,8} = 0.954$, n.s.; ZT $\times$ hormone, $F_{2,16} = 2.488$, n.s.; Fig. 5C). Relative gains in NREM sleep also did not differ between hormone treatment groups when considered over the whole 12-h dark phase ($t_9 = 0.820$, n.s.; Fig. 5D). Together, these data suggest that E2 mildly attenuated a SD-induced increase in NREM sleep in the mid-dark phase of recovery day 1.

3.3.4. Wake—Upon release from SD, E2-treated rats spent more time awake than oil-treated rats through the early- and mid-dark phase (ZT $\times$ hormone, $F_{2,16} = 16.911$, $p < 0.001$; Fig. 4C), consistent with the observed decreases in REM and NREM sleep duration in this group.

3.4. Recovery day 2

3.4.1. REM sleep—On recovery day 2, REM duration returned to levels similar to baseline in both groups; E2 did not affect REM sleep in the light phase (hormone, $F_{1,8} = 0.002$, n.s.; ZT $\times$ hormone, $F_{2,16} = 3.012$, n.s.), but reduced REM duration in the early- mid-dark phase compared to oil (ZT $\times$ hormone, $F_{2,16} = 14.146$, $p < 0.001$; Fig. 6A). Gain-loss ratios for the light and dark phases on the day after SD indicated minimal ($< 7\%$) changes in REM sleep during the light or dark phases with no effect of E2 (data not shown) indicating that spontaneous REM sleep had returned to baseline levels.

3.4.2. NREM sleep—There were no effects of hormone treatment on NREM sleep in the light phase (hormone, $F_{1,8} = 0.375$, n.s.; ZT $\times$ hormone, $F_{2,16} = 2.438$, n.s.). In the dark phase, there was a significant interaction between ZT and hormone treatment ($F_{2,16} = 12.828$, $p < 0.001$); E2-treated rats exhibited a near-significant trend towards less time in NREM sleep in the early-mid dark phase (ZT 12– ZT 20; posthoc, $p = 0.057$) and spent more time in NREM sleep in the late dark phase than oil-treated rats (ZT 20 – ZT 24; posthoc, $p = 0.022$). Gain-loss ratios on recovery day 2 indicated minimal ($< 8\%$) relative changes in NREM sleep from baseline during the light or dark phases with no effect of E2 (data not shown), suggesting that NREM recovery was largely completed by this time.

3.4.3. Wake—E2 did not influence waking in the light phase (hormone, $F_{1,8} = 0.279$, n.s.; ZT $\times$ hormone, $F_{2,16} = 0.451$, n.s.); however, E2 increased waking in the early- mid dark phase, while decreasing it in the late dark phase (ZT 20 – ZT 24) (ZT $\times$ hormone, $F_{2,16} = 15.919$, $p < 0.001$; Fig. 6C). This E2-dependent increase in waking complemented the observed alterations in REM and NREM sleep, indicating that on recovery day 2 E2-treated rats spent more time awake and less time asleep in the early- to mid-dark phase.

3.5. Sleep Architecture

Total bout number and average bout duration for the light and dark phases of each experimental day are summarized in Table 1. In the dark phase, E2 shortened the average REM bout duration compared to oil over all three experimental days (hormone, $F_{1,8} = 7.316$,

$p = 0.026$); E2-treated rats also exhibited a trend towards fewer REM bouts in the dark phase, as reported elsewhere (51) but the present comparison was not significant (hormone, $F_{1,8} = 2.264$, n.s.; ZT $\times$ hormone, $F_{2,16} = 0.968$, n.s.). There were no other significant effects of hormone on bout number or bout duration in the light phase or the dark phase of any day.

SD significantly increased total REM and NREM bout number (REM, $F_{2,16} = 20.5$, $p < 0.001$; NREM, $F_{2,16} = 6.030$, $p = 0.011$) and average bout duration (REM, $F_{2,16} = 46.436$, $p < 0.001$; NREM, $F_{2,16} = 4.796$, $p = 0.023$) on recovery day 1 compared to baseline and recovery day 2, whereas SD decreased average wake bout duration ($F_{2,16} = 7.707$, $p = 0.004$) on recovery day 1. SD thus temporarily increased REM and NREM sleep bout number and length during the recovery day 1 dark phase, with a return to baseline levels on Recovery day 2, with no further modulation of rebound sleep architecture by E2.

3.6. NREM delta power

At baseline, NREM delta power was elevated at lights-on and steadily declined over the light phase (Fig. 7A). E2 did not affect NREM delta power during baseline spontaneous sleep in the light phase (hormone, $F_{1,8} = 1.055$, n.s.; ZT $\times$ hormone, $F_{2,16} = 0.698$, n.s) or the dark phase (hormone, $F_{1,8} = 0.102$, n.s.; ZT $\times$ hormone, $F_{2,16} = 1.617$, n.s.).

NREM delta power decreased during SD in E2- and oil-treated rats. Upon release from SD, delta power increased in both treatment groups, but E2 appeared to attenuate the magnitude of this increase and prolong its duration, compared to oil (ZT $\times$ hormone, $F_{2,16} = 6.537$, $p = 0.008$; Fig. 7B). Specifically, NREM delta power declined in the late dark phase (ZT 20 – ZT 24) of recovery day 1 in oil-treated rats and E2 prevented this decline.

On recovery day 2, NREM delta power returned to baseline levels; E2 did not affect NREM delta power during spontaneous sleep in the light phase (hormone, $F_{1,8} = 3.529$, n.s.; ZT $\times$ hormone, $F_{2,16} = 0.742$, n.s) or the dark phase (hormone, $F_{1,8} = 0.105$, n.s.; ZT $\times$ hormone, $F_{2,16} = 1.157$, n.s.; Fig. 7C).

4. Discussion

In intact female rats, spontaneous REM and NREM sleep are suppressed at proestrus, when circulating E2 levels are high, without exhibiting a compensatory rebound (24,25). Based on this, we hypothesized that E2 suppresses recovery sleep during the rat's active (dark) phase. In the present study, OVX rats exposed to low physiological doses of E2 versus oil vehicle (1) suppressed spontaneous REM sleep in the dark phase before and after SD, and (2) attenuated REM rebound during the recovery (i.e. in recovery day 1). E2 did not suppress NREM sleep or increase wakefulness at baseline (three days after capsule implant), but it did attenuate NREM recovery, and decreased NREM sleep and increased waking in the dark phase five days after implant. These results demonstrate first, that E2 alters the dynamics of REM and, to a lesser extent, NREM recovery sleep in the dark phase. Second, these data suggest that REM sleep is more sensitive to suppression by E2, as these effects appeared much earlier in the experiment compared to effects on NREM and waking, and remained stable for the duration of the study.

In intact female rats, REM and NREM sleep suppression is highest at proestrus, when circulating E2 levels increase 3- to 5-fold within 24 h (20,24,25). Reported peak serum E2 concentrations vary between 50 pg/ml to 150 pg/mL at proestrus (49,52–54). Our hormone treatment unexpectedly resulted in circulating E2 concentrations of ~30 pg/mL, somewhat lower than these reported peak values. This low physiological dose nevertheless altered spontaneous and recovery sleep-wake patterns compared to oil. The observed E2 concentrations are consistent with those reported by Deurveilher et al (2009), who also

report baseline REM suppression at low physiological doses of E2 after a prolonged exposure (10–14 days from capsule implant). Thus, in OVX rats E2 is capable of reducing REM sleep at lower concentrations than typically seen in intact cycling rats, and does so within three days of initial exposure.

Both E2- and oil-treated rats increased REM sleep duration during recovery. However, E2 significantly attenuated this REM recovery compared to oil, whether measured by actual REM duration in the recovery period or by the relative increase over baseline REM sleep. Only a handful of studies to date have examined E2's effects on recovery sleep in rodents; to our knowledge, this constitutes the first description of E2-mediated suppression of REM rebound. These data suggest first, that E2 exerts similar suppressive effects on spontaneous and recovery REM sleep. In intact female rats, estrous phase was reported to have no effect on REM recovery following 6 h SD (24); however, the published vigilance state data in that study do suggest increased wake and decreased REM recovery in the late proestrus day compared to estrus (see Table 2 in Schwierin et al, 1998). Notably, this recovery occurred during the proestrus light phase, when baseline REM sleep was also suppressed (24,25). Similarly, in mice E2 suppressed NREM recovery but did not suppress REM recovery (30), although the data do appear to show a small decrease in REM sleep in the dark phase following SD. While not statistically significant, these trends are consistent with the idea that E2 exerts similar suppressive effects on spontaneous and recovery REM sleep.

In contrast, a recent study by Deurveilher et al (2009) reported that exogenous E2 was associated with increased REM sleep relative to baseline following 6 h SD in OVX rats. The authors report a significant reduction in baseline REM sleep in E2-treated animals compared to the oil-treated controls (~35%). However, recovery sleep following 6 h of SD was not significantly different between the two groups, suggesting that the relative increase in REM sleep may thus reflect the much larger suppression of baseline sleep by E2 alone, which we observed as well, rather than an effect on recovery sleep alone (see Fig. 2B, absolute and 5B, relative REM recovery in ref. 42) (42). The decision to use a 12 h SD in the present study, compared to a 6 h SD, could have contributed to the observed differences in results by increasing the magnitude of REM rebound in oil-treated rats while E2 kept the rebound shorter, resulting in a larger difference between the two groups in the 12 h condition than in a similar 6 h condition.

Second, in the present study, hormone-dependent differences in REM recovery were specific to the dark phase; REM sleep following the dark phase portion of recovery returned to baseline values in this study (see recovery day 2, light phase; Fig. 6A) as well as in Deurveilher et al (2009). Because of this, the aggregation of light and dark into a single recovery period in Deurveilher et al's study may have blunted differences in recovery sleep specific to the dark phase. In this context, it is notable that a follow-up analysis of the same animals revealed that E2 attenuated the number of REM episodes within a 12 h recovery period limited to the dark phase compared to oil (51). While we observed E2-mediated changes in REM episode duration, not number, and only observed them at baseline, these effects both point towards attenuation of REM sleep by E2 overall. Together, these data suggest a powerful, phase-specific influence of E2 on REM recovery.

In the present study, E2 suppressed NREM sleep compared to oil controls, but only in the recovery phase and not the baseline. This occurrence of E2-mediated NREM sleep suppression was accompanied by a significant increase in wake. One interpretation for the delay in E2-mediated NREM suppression may be the temporal and/or dose dependent dynamics of sleep suppression by exogenous hormones. An endogenous pulsatile E2 exposure could be more effective for modulating sleep-wake state in the short term than the static increase provided by capsules, as is the case for hormone facilitation of reproductive

behavior (55–57). Moreover, at the low dose used here a longer exposure may be necessary to elicit this aspect of sleep regulation. Two weeks of E2 exposure suppresses NREM sleep (30,42), and E2-induced waking is dose-dependent (15,19). Further work is needed to evaluate whether E2 replacement modeling the endogenous duration (24–48 h) and peak concentrations (~50 – 150 pg/mL) of the periovulatory E2 profile more effectively mimics the sleep phenotype of proestrus rats.

At present, little is known about how E2 modulates sleep, particularly REM sleep. Sleep, in part, is a homeostatically regulated behavior where the drive (or need) for sleep proportionally increases with the time spent out of sleep (i.e., in wake) (36,58,59). While this homeostasis is most closely associated with NREM sleep, REM sleep is homeostatically regulated independently of NREM sleep (38,39). Our current finding that E2 attenuates REM recovery sleep, in conjunction with our previous findings that REM sleep is suppressed and does not rebound during the proestrus day (25), suggests two possibilities.

First, E2 could attenuate the homeostatic drive for REM sleep, resulting in a reduced propensity for REM sleep. However, several studies report that intact female rats experience a REM rebound in the light phase of estrus (the day after proestrus), when circulating E2 levels are decreasing (20,24). Previously, we did not observe a statistically significant increase in REM sleep at estrus, although REM duration was somewhat elevated compared to diestrus, consistent with a rebound at this time (see Fig. 6 in ref. 25) (25). It is not known whether OVX rats treated with exogenous E2 would exhibit a REM rebound upon washout of the hormone; future work could address this question using injections of a rapidly-clearing estrogen, rather than capsules.

Second, E2 could directly suppress REM sleep independently of homeostatic factors. One possible mechanism for E2 to suppress sleep is by acting on the circadian sleep-wake rhythm. In humans, the intrinsic period of the circadian pacemaker is shorter in women than in men (60), and E2 replacement in postmenopausal women enhances daily rhythms in serum cortisol (61). In rodents, E2 modulates circadian rhythms in locomotor activity (16,62) and gene expression (63,64); when administered with progesterone, E2 has also been shown to phase-delay circadian rhythms in explanted uterine tissue (65), suggesting a direct role in modulating rhythms in the periphery. The circadian clock is thus sensitive to regulation by E2, as well as being key to normal reproductive function (66)(67,68).

The circadian pacemaker actively promotes both sleep and waking in rats during the day and night respectively (69), modulates the homeostatic response to sleep deprivation (40,41,70) and tightly regulates REM sleep timing across the subjective day in rats and in humans (71–73). If the pacemaker is responsive to E2, as has been suggested by functional data as well as the presence of estrogen receptors in the SCN (74), then it is possible that E2 could influence circadian sleep-wake rhythms, as it has been shown to influence other rhythms. A small but growing literature supports this idea. OVX attenuates, and exogenous E2 restores, daily rhythms in Fos expression in the VLPO and in the dorsomedial suprachiasmatic nucleus (SCN) (75), suggesting that E2 acts simultaneously on brain regions responsible for regulating sleep and circadian rhythms, respectively. Intact female rats tend to show the greatest suppression of REM sleep around the light-to-dark transition, when wakefulness normally starts to predominate over sleep (20,25,76), supporting a time-of-day quality to E2's effects on sleep. Similarly, OVX rats given E2 replacement showed increased brief awakening coupled with decreases in NREM episode duration and REM episode number in the active (dark) phase (51). In the present study, E2 suppressed spontaneous sleep and promoted waking during the active (dark) phase, suppressed REM and NREM recovery sleep in the dark phase, and acted preferentially on REM sleep, supporting the hypothesis that E2 acts to consolidate or otherwise strengthen the circadian sleep-wake rhythm.

At present, it is not possible to exclude either a homeostatic or a circadian mechanism of action for E2 on the basis of the current data alone. If E2 does modulate the circadian sleep-wake drive in female rats, then E2-treated rats should respond to SD differently when deprivation ends in the light phase, when the circadian clock promotes sleep and/or suppresses waking, versus the dark phase, when waking is favored. More specifically, E2 should increase recovery sleep in the light phase compared to oil, while attenuating recovery sleep in the dark phase. Conversely, if E2 acts on homeostatic mechanisms that regulate REM and/or NREM sleep, E2 should attenuate recovery sleep regardless of what time of day the deprivation ends. We are currently testing this working hypothesis.

In contrast to the profound attenuation of baseline and recovery REM sleep, NREM sleep was only mildly affected by E2. As previously mentioned, the homeostatic drive to sleep increases in proportion to the duration of prior wakefulness. This relationship has been extensively modeled (36) and most accurately predicts SD-induced increases in NREM sleep duration and intensity, as assayed by NREM delta power (77,78). Although the current SD failed to completely deprive rats of NREM sleep over the 12h light phase, the amount of residual NREM during SD was similar in both groups. Furthermore, this admittedly partial NREM deprivation elicited a positive NREM rebound when recovery sleep was normalized to baseline in both treatment groups suggesting that our gentle handling manipulation increased NREM pressure to a similar extent in both groups.

During recovery from SD, E2 attenuated NREM duration in the mid-dark phase, and attenuated NREM delta power as previously reported (42,51). The fact that E2 suppressed both NREM recovery duration and NREM delta power, despite undergoing a comparable loss in NREM sleep during SD, suggests that E2 could alter NREM sleep efficiency (42,51), although in that case E2 would also be expected to decrease the NREM gain-loss ratio, which it did not. On the subsequent day (recovery day 2) NREM delta power was unaffected, while NREM duration was attenuated by E2. In intact rats, baseline NREM delta power was decreased at proestrus but recovery delta power was unaffected by estrus phase (24). It is unclear why reductions in NREM delta power manifested differently in these studies (eg. spontaneous vs recovery sleep). E2 may alter NREM efficiency (42,51), or simply suppress NREM sleep. Nevertheless, this suppression is likely to involve the sleep-promoting ventrolateral preoptic area (VLPO) (33,79) as E2 has been reported to decrease Fos expression (25,34), which is consistent with the NREM suppression.

5. Conclusions

E2's role as an important regulator of sleep-wake state is only now beginning to emerge. Exogenous ovarian hormones are associated with subjectively improved sleep quality in menopausal women (11,80) and with increased NREM sleep in younger healthy women (81), consistent with the idea that E2 consolidates the daily sleep-wake rhythm. Furthermore, the fact that sleep disruption in women frequently accompanies large alterations in the circulating ovarian hormone milieu suggests that these hormones play an important role in regulating sleep-wake state. However, very few studies to date have attempted to tease apart the mechanisms by which exogenous ovarian hormones impact sleep in rodent models. Here, we show that exposure to E2 at low physiological doses suppresses spontaneous and recovery REM sleep in the dark phase, with smaller, similarly phase-dependent effects on NREM sleep and waking. Moreover, we show for the first time that these effects can be observed in as little as five days, or in the case of REM sleep, three days, after initiating E2 exposure. These data suggest that the alterations in spontaneous and recovery sleep in intact, cycling rats that we and others have previously reported are likely to be mediated by E2, are highly preferential for REM sleep, and support the working hypothesis that E2 regulates sleep by acting on the circadian sleep-wake rhythm.

Highlights

Low physiological doses of estradiol suppress nighttime sleep in female rats. Estradiol attenuates recovery of REM and NREM sleep from sleep deprivation. REM sleep is more sensitive to suppression by estradiol than NREM sleep. Estradiol may consolidate circadian sleep-wake rhythms in female rats.

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References

1. National Sleep Foundation. 2008. 2007 Women and Sleep | National Sleep Foundation - Information on Sleep Health and Safety.
2. Zhang B, Wing Y. Sex differences in insomnia: a meta-analysis. *Sleep*. 2006; 29(1):85–93. [PubMed: 16453985]
3. McKnight-Eily LR, Liu Y, Perry GS, Presley-Cantrell LR, Strive TW, Lu H, et al. Perceived Insufficient Rest or Sleep Among Adults — United States, 2008. *Morbidity and Mortality Weekly Report*. 2009 Nov. 5; 58(42):1175–1179. [PubMed: 19875979]
4. Moline M, Broch L, Zak R, Gross V. Sleep in women across the life cycle from adulthood through menopause. *Sleep Med Rev*. 2003 Apr. 1; 7(2):155–177. [PubMed: 12628216]
5. Baker FC, Driver HS. Circadian rhythms, sleep, and the menstrual cycle. *Sleep Med*. 2007 Sep.; 8(6):613–622. [PubMed: 17383933]
6. Hagenauer MH, Perryman JJ, Lee TM, Carskadon MA. Adolescent changes in the homeostatic and circadian regulation of sleep. *Dev. Neurosci*. 2009; 31(4):276–284. [PubMed: 19546564]
7. Sowers MR, Zheng H, Kravitz HM, Matthews K, Bromberger JT, Gold EB, et al. Sex Steroid Hormone Profiles are Related to Sleep Measures from Polysomnography and the Pittsburgh Sleep Quality Index. *Sleep*. 2008 Oct. 1; 31(10):1339. [PubMed: 18853931]
8. Shechter A, Varin F, Boivin DB. Circadian variation of sleep during the follicular and luteal phases of the menstrual cycle. *Sleep*. 2010 May 1; 33(5):647–656. [PubMed: 20469807]
9. Dzaja A, Arber S, Hislop J, Kerkhofs M, Kopp C, Pollmacher T, et al. Women's sleep in health and disease. *J Psychiatr Res*. 2005; 39:55–76. [PubMed: 15504424]
10. Parry BL, Newton RP. Chronobiological basis of female-specific mood disorders. *Neuropsychopharmacology*. 2001 Nov. 1; 25(5 Suppl):S102–S108. [PubMed: 11682284]
11. Gambacciani M, Ciaponi M, Cappagli B, Monteleone P, Benussi C, Bevilacqua G, et al. Effects of low-dose, continuous combined estradiol and norethisterone acetate on menopausal quality of life in early postmenopausal women. *Maturitas*. 2003 Feb. 25; 44(2):157–163. [PubMed: 12590012]
12. Murphy PJ, Campbell SS. Sex hormones, sleep, and core body temperature in older postmenopausal women. *Sleep*. 2007 Dec. 1; 30(12):1788–1794. [PubMed: 18246988]
13. Borbely A. Sleep mechanisms. *Sleep and Biological Rhythms*. 2004; 2:S67–S68.
14. Lightfoot JT. Sex hormones' regulation of rodent physical activity: a review. *Int J Biol Sci*. 2008; 4(3):126–132. [PubMed: 18449357]
15. Finger FW. Estrus and general activity in the rat. *J Comp Physiol Psychol*. 1969 Jul. 1; 68(3):461–466. [PubMed: 5817704]
16. Albers HE, Gerall AA, Axelsson JF. Effect of reproductive state on circadian periodicity in the rat. *Physiol Behav*. 1981; 26(1):21–25. [PubMed: 7195042]
17. Gorzsek JF, Hendrickson KC, Forstner JP, Rixen JL, Moran AL, Lowe DA. Estradiol and tamoxifen reverse ovariectomy-induced physical inactivity in mice. *Med Sci Sports Exerc*. 2007 Feb. 1; 39(2):248–256. [PubMed: 17277588]
18. Ogawa S, Chan J, Gustafsson J, Korach KS, Pfaff DW. Estrogen increases locomotor activity in mice through estrogen receptor alpha: specificity for the type of activity. *Endocrinology*. 2003; 144(1):230–239. [PubMed: 12488349]

19. Fahrbach SE, Meisel RL, Pfaff DW. Preoptic implants of estradiol increase wheel running but not the open field activity of female rats. *Physiol Behav.* 1985 Dec. 1; 35(6):985–992. [PubMed: 4095192]
20. Colvin GB, Whitmoyer DI, Lisk RD, Walter DO, Sawyer CH. Changes in sleep-wakefulness in female rats during circadian and estrous cycles. *Brain Res.* 1968 Feb. 1; 7(2):173–181. [PubMed: 5689033]
21. Yamaoka S. Participation of limbic-hypothalamic structures in circadian rhythm of slow wave sleep and paradoxical sleep in the rat. *Brain Res.* 1978 Aug. 4; 151(2):255–268. [PubMed: 209863]
22. Kleinlogel H. The female rat's sleep during oestrous cycle. *Neuropsychobiology.* 1983; 10(4):228–237. [PubMed: 6687132]
23. Zhang JH, Sampogna S, Morales FR, Chase MH. Distribution of hypocretin (orexin) immunoreactivity in the feline pons and medulla. *Brain Res.* 2004 Jan. 9; 995(2):205–217. [PubMed: 14672810]
24. Schwierin B, Borbely AA, Tobler I. Sleep homeostasis in the female rat during the estrous cycle. *Brain Res.* 1998 Nov. 16; 811(1–2):96–104. [PubMed: 9804908]
25. Hadjimarkou M, Benham R, Schwarz J, Holder M, Mong J. Estradiol suppresses rapid eye movement sleep and activation of sleep-active neurons in the ventrolateral preoptic area. *Eur J Neurosci.* 2008 Apr. 1; 27(7):1780–1792. [PubMed: 18371078]
26. Koehl M, Battle SE, Turek FW. Sleep in female mice: a strain comparison across the estrous cycle. *Sleep.* 2003 May 1; 26(3):267–272. [PubMed: 12749544]
27. Branchey M, Branchey L, Nadler RD. Effects of estrogen and progesterone on sleep patterns of female rats. *Physiol Behav.* 1971 Jun.; 6(6):743–746. [PubMed: 4337177]
28. Yamaoka S. Modification of circadian sleep rhythms by gonadal steroids and the neural mechanisms involved. *Brain Res.* 1980 Mar. 10; 185(2):385–398. [PubMed: 6101987]
29. Paul KN, Dugovic C, Turek FW, Laposky AD. Diurnal sex differences in the sleep-wake cycle of mice are dependent on gonadal function. *Sleep.* 2006 Sep. 1; 29(9):1211–1223. [PubMed: 17040009]
30. Paul KN, Laposky AD, Turek FW. Reproductive hormone replacement alters sleep in mice. *Neurosci Lett.* 2009 Oct. 9; 463(3):239–243. [PubMed: 19647784]
31. Colvin GB, Whitmoyer DI, Sawyer CH. Circadian sleep-wakefulness patterns in rats after ovariectomy and treatment with estrogen. *Exp Neurol.* 1969 Dec. 1; 25(4):616–625. [PubMed: 5391423]
32. Scammell TE, Gerashchenko D, Urade Y, Onoe H, Saper C, Hayaishi O. Activation of ventrolateral preoptic neurons by the somnogen prostaglandin D2. *Proc Natl Acad Sci USA.* 1998; 95(13):7754–7759. [PubMed: 9636223]
33. Sherin JE, Shiromani PJ, McCarley RW, Saper CB. Activation of Ventrolateral Preoptic Neurons During Sleep. *Science.* 1996 Jan. 12; 271(5246):216–219. [PubMed: 8539624]
34. Deurveilher S, Cumyn EM, Peers T, Rusak B, Semba K. Estradiol replacement enhances sleep deprivation-induced c-Fos immunoreactivity in forebrain arousal regions of ovariectomized rats. *Am J Physiol Regul Integr Comp Physiol.* 2008 Oct. 1; 295(4):R1328–R1340. [PubMed: 18753261]
35. Qu W, Huang Z, Xu X, Aritake K, Eguchi N, Nambu F, et al. Lipocalin-type prostaglandin D synthase produces prostaglandin D2 involved in regulation of physiological sleep. *Proc Natl Acad Sci USA.* 2006 Nov. 21; 103(47):17949–17954. [PubMed: 17093043]
36. Borbely AA. A two process model of sleep regulation. *Hum Neurobiol.* 1982; 1(3):195–204. [PubMed: 7185792]
37. Borbely AA, Achermann P. Concepts and models of sleep regulation: an overview. *Journal of Sleep Research.* 1992 Jun.; 1(2):63–79. [PubMed: 10607028]
38. Ocampo-Garcés A, Molina E, Rodríguez A, Vivaldi EA. Homeostasis of REM sleep after total and selective sleep deprivation in the rat. *J Neurophysiol.* 2000 Nov. 1; 84(5):2699–2702. [PubMed: 11068012]

39. Shea JL, Mochizuki T, Sagvaag V, Aspevik T, Bjorkum AA, Datta S. Rapid eye movement (REM) sleep homeostatic regulatory processes in the rat: changes in the sleep-wake stages and electroencephalographic power spectra. *Brain Res.* 2008 Jun. 5.1213:48–56. [PubMed: 18455709]
40. Wurts SW, Edgar DM. Circadian and homeostatic control of rapid eye movement (REM) sleep: promotion of REM tendency by the suprachiasmatic nucleus. *J Neurosci.* 2000 Jun. 1; 20(11): 4300–4310. [PubMed: 10818165]
41. Kas MJ, Edgar DM. Circadian timed wakefulness at dawn opposes compensatory sleep responses after sleep deprivation in *Octodon degus*. *Sleep.* 1999 Dec. 15; 22(8):1045–1053. [PubMed: 10617165]
42. Deurveilher S, Rusak B, Semba K. Estradiol and progesterone modulate spontaneous sleep patterns and recovery from sleep deprivation in ovariectomized rats. *Sleep.* 2009 Jul. 1; 32(7):865–877. [PubMed: 19639749]
43. Moore-Ede, M.; Sulzman, F.; Fuller, C. The clocks that time us : physiology of the circadian timing system. Cambridge MA: Harvard University Press; 1982.
44. Wise PM, Camp-Grossman P, Barraclough CA. Effects of Estradiol and Progesterone on Plasma Gonadotropins, Prolactin, and LHRH in Specific Brain Areas of Ovariectomized Rats. *Biology of Reproduction.* 1981; 24:820–830. [PubMed: 7018601]
45. Dubal DB, Kashon ML, Pettigrew LC, Ren JM, Finklestein SP, Rau SW, et al. Estradiol protects against ischemic injury. *J. Cereb. Blood Flow Metab.* 1998 Nov.; 18(11):1253–1258. [PubMed: 9809515]
46. Ström JO, Theodorsson E, Holm L, Theodorsson A. Different methods for administering 17beta-estradiol to ovariectomized rats result in opposite effects on ischemic brain damage. *BMC Neurosci.* 2010; 11:39. [PubMed: 20236508]
47. Ström JO, Theodorsson E, Theodorsson A. Order of magnitude differences between methods for maintaining physiological 17β-oestradiol concentrations in ovariectomized rats. *Scand J Clin Lab Invest.* 2008 Jan.; 68(8):814–822. [PubMed: 18821130]
48. Blutstein T, Baab P, Zielke H, Mong J. Hormonal modulation of amino acid neurotransmitter metabolism in the arcuate nucleus of the adult female rat: a novel action of estradiol. *Endocrinology.* 2009 Jul. 1; 150(7):3237–3244. [PubMed: 19299450]
49. Nequin LG, Alvarez J, Schwartz NB. Measurement of Serum Steroid and Gonadotropin Levels and Uterine and Ovarian Variables throughout 4 Day and 5 Day Estrous Cycles in the Rat. *Biology of Reproduction.* 1979; 20:659–670. [PubMed: 572241]
50. Carter, C. Behavioral Endocrinology, Second Edition. second edition. The MIT Press; 2002.
51. Deurveilher S, Rusak B, Semba K. Female reproductive hormones alter sleep architecture in ovariectomized rats. *Sleep.* 2011; 34(4):519–530. [PubMed: 21461331]
52. Butcher RL, Collins WE, Fugo NW. Plasma concentration of LH, FSH, prolactin, progesterone and estradiol-17beta throughout the 4-day estrous cycle of the rat. *Endocrinology.* 1974 Jun.; 94(6):1704–1708. [PubMed: 4857496]
53. Smith MS, Freeman ME, Neill JD. The control of progesterone secretion during the estrous cycle and early pseudopregnancy in the rat: prolactin, gonadotropin and steroid levels associated with rescue of the corpus luteum of pseudopregnancy. *Endocrinology.* 1975; 96(1):219–226. [PubMed: 1167352]
54. Henderson SR, Baker C, Fink G. Oestradiol-17beta and pituitary responsiveness to luteinizing hormone releasing factor in the rat: a study using rectangular pulses of oestradiol-17beta monitored by non-chromatographic radioimmunoassay. *J Endocrinol.* 1977 Jun.; 73(3):441–453. [PubMed: 327006]
55. Clark AS, Roy EJ. Behavioral and cellular responses to pulses of low doses of estradiol-17β. *Physiol Behav.* 1983 Apr.; 30(4):561–565. [PubMed: 6878455]
56. Clark AS, Roy EJ. Effective intervals for the administration of estradiol pulses and the induction of sexual behavior in female rats. *Physiol Behav.* 1987; 39(5):665–667. [PubMed: 3588717]
57. Becker JB, Arnold AP, Berkley KJ, Blaustein JD, Eckel LA, Hampson E, et al. Strategies and methods for research on sex differences in brain and behavior. *Endocrinology.* 2005 Apr. 1; 146(4):1650–1673. [PubMed: 15618360]

58. Borbely, AA. Sleep: Circadian rhythm versus recovery process. Amsterdam: Elsevier North Holland Biomedical Press; 1980.
59. Borbely AA, Achermann P. Sleep homeostasis and models of sleep regulation. *J Biol Rhythms*. 1999; 14(6):557–568. [PubMed: 10643753]
60. Duffy JF, Cain SW, Chang A, Phillips AJK, Münch MY, Gronfier C, et al. Quantification of Behavior Sackler Colloquium: Sex difference in the near-24-hour intrinsic period of the human circadian timing system. *Proc Natl Acad Sci USA*. 2011 May 2. epub ahead of print.
61. Gudmundsson A, Goodman B, Lent S, Barczy S, Grace A, Boyle L, et al. Effects of estrogen replacement therapy on the circadian rhythms of serum cortisol and body temperature in postmenopausal women. *Exp Gerontol*. 1999; 34(6):809–818. [PubMed: 10579640]
62. Albers H. Gonadal hormones organize and modulate the circadian system of the rat. *Am J Physiol*. 1981 Jul. 1; 241(1):R62–R66. [PubMed: 7246802]
63. Nakamura T, Moriya T, Inoue S, Shimazoe T, Watanabe S, Ebihara S, et al. Estrogen differentially regulates expression of Per1 and Per2 genes between central and peripheral clocks and between reproductive and nonreproductive tissues in female rats. *J Neurosci Res*. 2005; 82(5):622–630. [PubMed: 16273538]
64. Nakamura TJ, Sellix MT, Menaker M, Block GD. Estrogen directly modulates circadian rhythms of PER2 expression in the uterus. *Am J Physiol Endocrinol Metab*. 2008 Nov.; 295(5):E1025–E1031. [PubMed: 18728223]
65. Nakamura TJ, Sellix MT, Kudo T, Nakao N, Yoshimura T, Ebihara S, et al. Influence of the estrous cycle on clock gene expression in reproductive tissues: effects of fluctuating ovarian steroid hormone levels. *Steroids*. 2010 Mar.; 75(3):203–212. [PubMed: 20096720]
66. Banik UK, Herr F. Effects of medroxyprogesterone acetate and chlorpromazine on ovulation and vaginal cytology in a strain of 4-day cyclic rats. *Can. J. Physiol. Pharmacol*. 1969 Jun.; 47(6):573–575. [PubMed: 5816160]
67. Miller B, Olson S, Turek FW, Levine J, Horton T, Takahashi J. Circadian clock mutation disrupts estrous cyclicity and maintenance of pregnancy. *Curr Biol*. 2004 Aug. 10; 14(15):1367–1373. [PubMed: 15296754]
68. Mahoney MM. Shift work, jet lag, and female reproduction. *Int J Endocrinol*. 2010; 2010:813764. [PubMed: 20224815]
69. Mistlberger R. Circadian regulation of sleep in mammals: Role of the suprachiasmatic nucleus. *Brain Res Rev*. 2005; 49(3):429–454. [PubMed: 16269313]
70. Yassenkov R, Deboer T. Circadian regulation of sleep and the sleep EEG under constant sleep pressure in the rat. *Sleep*. 2010 May 1; 33(5):631–641. [PubMed: 20469805]
71. Czeisler CA, Weitzman ED, Moore-Ede MC, Zimmerman JC, Knauer R. Human sleep: its duration and organization depend on its circadian phase. *Science*. 1980 Dec. 12; 210(4475):1264–1267. [PubMed: 7434029]
72. Cambras T, Weller JR, Anglès-Pujorès M, Lee ML, Christopher A, Díez-Noguera A, et al. Circadian desynchronization of core body temperature and sleep stages in the rat. *Proc Natl Acad Sci USA*. 2007 May 1; 104(18):7634–7639. [PubMed: 17452631]
73. Lee ML, Swanson BE, la Iglesia de HO. Circadian timing of REM sleep is coupled to an oscillator within the dorsomedial suprachiasmatic nucleus. *Curr Biol*. 2009 May 26; 19(10):848–852. [PubMed: 19375313]
74. Vida B, Hrabovszky E, Kalamatianos T, Coen CW, Liposits Z, Kallo I. Oestrogen receptor alpha and beta immunoreactive cells in the suprachiasmatic nucleus of mice: distribution, sex differences and regulation by gonadal hormones. *J Neuroendocrinol*. 2008 Nov.; 20(11):1270–1277. [PubMed: 18752649]
75. Peterfi Z, Churchill L, Hajdu I, Obal F Jr, Krueger JM, Parducz A. Fos-immunoreactivity in the hypothalamus: dependency on the diurnal rhythm, sleep, gender, and estrogen. *Neuroscience*. 2004; 124(3):695–707. [PubMed: 14980739]
76. Schwierin B, Borbely AA, Tobler I. Prolonged effects of 24-h total sleep deprivation on sleep and sleep EEG in the rat. *Neurosci Lett*. 1999 Feb. 12; 261(1–2):61–64. [PubMed: 10081927]
77. Borbely AA, Neuhaus H. Sleep-Deprivation: Effects on sleep and EEG in the rat. *J Comp Physiol*. 1979; 133:71–87.

78. Vyazovskiy VV, Achermann P, Tobler I. Sleep homeostasis in the rat in the light and dark period. *Brain Res Bull.* 2007 Sep. 14; 74(1–3):37–44. [PubMed: 17683787]
79. Lu J, Greco M, Shiromani PJ, Saper C. Effect of lesions of the ventrolateral preoptic nucleus on NREM and REM sleep. *J Neurosci.* 2000; 20(10):3830–3842. [PubMed: 10804223]
80. Kravitz HM, Zhao X, Bromberger JT, Gold EB, Hall MH, Matthews KA, et al. Sleep disturbance during the menopausal transition in a multi-ethnic community sample of women. *Sleep.* 2008 Jul. 1; 31(7):979–990. [PubMed: 18652093]
81. Baker FC, Mitchell D, Driver HS. Oral contraceptives alter sleep and raise body temperature in young women. *Pflugers Arch.* 2001 Aug. 1; 442(5):729–737. [PubMed: 11512029]

Figure 1. Timeline of the experiment. White bars represent light phase and dark bars represent dark phase.

Figure 2. Serum E2 concentrations taken from E2- and oil-treated OVX rats at the end of EEG recording. *, $p < 0.05$ vs oil.

Figure 3.

Total minutes spent in REM (A–B), NREM (C–D) and Wake (E–F) in the baseline day in OVX rats treated with E2 (dark lines) or oil (light lines). Data are plotted as a 12 h sums for the light and dark phases (A, C, E) and as 4 h sums (B, D, F) to illustrate the distribution of each vigilance state across the day. LD cycle is indicated on X-axis of each graph; time (Zeitgeber time (ZT), where ZT 0 = lights-on) is indicated below LD bar (B, D, F). *, $p < 0.05$ vs oil.

Figure 4. Total minutes spent in REM (A), NREM (B) and Wake (C) on the SD/recovery day in OVX rats treated with E2 (dark lines) or oil (light lines). Data are plotted as 4 h sums as described above. *, $p < 0.05$ vs oil.

Figure 5.

Gain-loss ratios for REM (A–B) and NREM sleep (C–D) in the 12 h dark phase following SD in OVX rats treated with E2 (dark lines) or oil (light lines). Gain-loss ratios were calculated according to the formula (sleep gained over baseline divided by sleep lost during recovery). Data are plotted as 4 h sums (A, C) and as 12 h sums for the dark phase (B, D) to illustrate the changes over time relative to baseline sleep and the net change over baseline. *, $p < 0.05$ vs oil.

Figure 6.

Figure 4: Total minutes spent in REM (A), NREM (B) and Wake (C) on recovery day 2 (the day after SD) in OVX rats treated with E2 (dark lines) or oil (light lines). Data are plotted as 4 h sums as described above. *, $p < 0.05$ vs oil.

Figure 7. NREM delta power as a percentage of sleep EEG spectrum in OVX rats treated with E2 (dark lines) or oil (light lines). Data for baseline day (A), SD/recovery day (B) and recovery day 2 (C) are averaged in 4 h bins. LD cycle is indicated on X-axis. #, $p < 0.05$ vs other time points. D phase only

Table

	Baseline			SD			Recovery 1			Recovery 2		
	E2	Oil	E2	E2	Oil	E2	E2	Oil	E2	Oil	E2	Oil
REM	176.6 (13.7)	192.6 (18.9)	41.4 (17.6)	17.8 (4.9)	23.2 (16.5)	88.6 [#] (10.6)	116.0 [#] (10.2)	180.0 (14.1)	39.6 (10.8)	58.2 (8.4)		
NREM	267.2 (21.9)	284.0 (19.8)	134.0 (43)	146.0 (34.9)	148.6 (44.4)	181.0 [#] (17.1)	172.2 [#] (16)	274.8 (14.4)	117.0 (32.8)	123.0 (19.6)		
Wake	124.0 (17)	122.6 (4.4)	117.2 (29.7)	150.4 (30.3)	151.2 (32)	137.8 (10.5)	113.0 (10.9)	137.8 (12.7)	121.0 (23)	106.2 (15.8)		
REM	38.2 ^{###} (4.3)	35.2 ^{###} (2.9)	15.2 [*] (3)	14.4 (4.4)	7.8 (2.9)	36.7 [#] (4.7)	47.2 [#] (3.3)	31.6 (4.2)	18.3 [*] (1.6)	30.0 (4)		
NREM	105.4 (9.1)	97.1 (5.4)	57.3 (8.7)	38.5 (9.7)	42.8 (9.9)	61.5 [#] (6.7)	76.2 [#] (4.2)	101.1 (5.1)	52.2 (5.6)	60.3 (3.7)		
Wake	106.5 (15.9)	85.5 (9.3)	511.4 (134.5)	476.9 (178.2)	531.9 (205.4)	250.7 [#] (41.6)	300.0 [#] (71.1)	90.4 (8.5)	475.3 (121.7)	432.5 (92.2)		

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[#]p<0.05 vs Baseline, Recovery 2

[#]p<0.05 vs Recovery 2

[#]p<0.05 vs Oil