Ovarian hormones promote recovery from sleep deprivation by increasing sleep intensity in middle-aged ovariectomized rats

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ABSTRACT

Sleep disturbances are commonly associated with menopause. Hormone replacement therapy is often used to treat various menopausal symptoms, but its efficacy for improving sleep is a matter of debate. We addressed this question by using a rodent model of ovarian hormone loss and replacement in midlife. Middle-aged female rats were ovariectomized and implanted with capsules containing estradiol with or without progesterone, or oil. After two weeks, sleep/wake states were recorded polygraphically during a 24-h baseline period, followed by 6 h of sleep deprivation in the second half of the light phase, and a 24-h recovery period. During the baseline dark phase, hormone treatments increased wakefulness, and decreased non-rapid eye movement sleep (NREMS) by shortening NREMS episodes; however, NREMS EEG delta power or energy (cumulative power) was unaffected by combined hormones. Following sleep deprivation, all the groups showed NREMS and rapid eye movement sleep (REMS) rebounds, with similar relative increases from respective baseline levels. The increases in NREMS EEG delta power/energy during recovery were enhanced by combined hormones. These results from middle-aged ovariectomized rats indicate that replacement with estrogen with or without progesterone reduces baseline NREMS without affecting sleep intensity, particularly during the dark (active) phase, whereas following sleep deprivation the same hormone treatments do not affect the ability to increase NREMS or REMS, but treatment with both hormones, in particular, enhances the intensity of recovery sleep. These results support the usefulness of ovariectomized middle-aged rats as a model system to study the biological effects of hormone replacement on sleep regulation.

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Introduction

Poor sleep quality is one of the common symptoms reported by peri- and postmenopausal women (Dzaja et al., 2005; Polo-Kantola, 2011). Declining levels of ovarian steroid hormones, including estradiol (E) and progesterone (P), can directly affect sleep regulation or circadian rhythms of sleep, or trigger vasomotor symptoms (hot flashes and night sweats) and mood disturbances, which can indirectly affect sleep (Joffe et al., 2010; Polo-Kantola, 2011). Hormone replacement therapy (estrogens with or without progestin) can alleviate various menopausal symptoms (NAMS, 2012), but the efficacy of these therapies for improving sleep quality is controversial, with both positive and negative reports (Joffe et al., 2010; Polo-Kantola, 2011). The discrepancies between the reports may be

related to differences in the type, duration, and timing of initiation of hormone therapy. Given the large number of postmenopausal women experiencing sleep disturbances and receiving hormone therapy (NAMS, 2012; Polo-Kantola, 2011), and the association between poor sleep quality and increased morbidity and mortality (Broussard et al., 2012; Luyster et al., 2012), more studies are needed to understand better how hormone replacement can affect sleep in women with declining levels of endogenous reproductive hormones.

Rodent models of hormone loss and replacement have proven useful for studying the impact of ovarian hormones on sleep, as well as underlying mechanisms, by allowing more strictly controlled manipulation of hormone levels than is possible in humans (Mong et al., 2011). Recently we treated young-adult, ovariectomized female rats with ovarian hormone implants for two weeks, and examined how these hormones influenced baseline sleep and recovery sleep after 6 h of sleep deprivation (Deurveilher et al., 2009, 2011). At baseline, treatments with E alone or combined with P reduced total sleep amount, by inhibiting both non-rapid eye movement sleep (NREMS) maintenance and rapid eye movement sleep (REMS) initiation, while promoting wakefulness, particularly during the dark (active)

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phase. However, when sleep pressure was increased after sleep deprivation, these hormone treatments facilitated REMS rebound and reduced the normal increase in the EEG measure of sleep intensity (Deurveilher et al., 2009, 2011). The inhibitory effect of E on baseline sleep is consistent with the previous findings in young ovariectomized rats (Barbe et al., 1970; Matsushima and Takeichi, 1990; Pawlyk et al., 2008). The facilitatory effect of E on recovery REMS contrasts with an inhibitory effect previously observed after 12 h of sleep deprivation in the light phase also in young ovariectomized rats (Schwartz and Mong, 2011); the reason for this discrepancy is unclear.

While the reports in women are inconsistent (see above), there are several differences between the results from the rodent models and the positive findings reported in women. In particular, E replacement decreases total sleep amount in ovariectomized rats (see above), whereas it can increase total sleep time in menopausal women according to some previous studies (Polo-Kantola, 2011). One factor that could contribute to these inconsistencies is the life stages of subjects who participated in these studies: young rodents vs. middle-aged women. In fact, loss of ovarian function and/or hormonal replacement may have different effects in individuals of different ages, due to age-related changes in hormone receptor levels and functions (Chakraborty and Gore, 2004). Given that hormone replacement therapy is most frequently used to treat women at middle age and later (NAMS, 2012), middle-aged, as opposed to young, female rats might be a better animal model for assessing the controversial efficacy of hormone therapy in improving sleep quality in peri- and postmenopausal women.

To test this possibility, we examined the effects of E, either alone or in combination with P, on baseline sleep and recovery sleep after 6 h of sleep deprivation in middle-aged, ovariectomized female rats. As in our previous studies in young female rats (Deurveilher et al., 2009, 2011), hormone treatments were achieved with hormone-filled capsules implanted subcutaneously for two weeks. These capsules can produce stable levels of hormones for several weeks, allowing baseline (24 h), sleep deprivation (6 h), and subsequent recovery sleep (24 h) to occur against the same controlled hormonal background in each animal. Such stable conditions cannot be attained in gonad-intact, middle-aged (9–12 month old) female rats, in which hormonal levels fluctuate across either regular or irregular estrous cycles (LeFevre and McClintock, 1988; Lu et al., 1979). Based on our previous studies in young female rats (Deurveilher et al., 2009, 2011), we hypothesized that E either alone or in combination with P will modulate baseline sleep differently than recovery sleep after sleep deprivation in middle-aged, ovariectomized rats, with some similarities and differences to what has been observed in young, ovariectomized rats.

Materials and methods

Animals

A total of 23 middle-aged female Wistar rats ("retired breeders", 9–11 months old; Harlan Laboratories, Indianapolis, IN) were used. Upon arrival from the supplier, the animals were housed in pairs and maintained on a 12-h light:12-h dark cycle (lights on at 07:00 = Zeitgeber Time [ZT] 0) in a colony room at 23 ± 1 °C, with food and water available ad libitum, for a period of 2–4 weeks for acclimation before surgery. Animal handling protocols followed the guidelines of the Canadian Council on Animal Care and were approved by the Dalhousie University Committee on Laboratory Animals.

The rats were randomly assigned to three treatment groups: Oil (n = 7), E (n = 8), and E plus P (EP; n = 8). With the exception of the type of anesthetics used for surgery, the surgery, recordings, and data acquisition/analyses were performed as in our previous studies (Deurveilher et al., 2009, 2011).

Surgery

Initially, surgeries were performed using a ketamine-based anesthetic (72 mg/kg ketamine, 3.8 mg/kg xylazine, and 0.7 mg/kg acepromazine, i.p.). However, as the mortality rate was high (40%), subsequent surgeries were conducted using isoflurane anesthesia (4% for induction and ≤2% for maintenance). All rats were ovariectomized bilaterally to deplete endogenous ovarian hormones. The uterine horns were pulled out of the abdominal cavity through a midline incision, and the ovaries were excised. Rats were then implanted with oil- or hormone-filled capsules, depending on the treatment group. Each rat in the Oil and E groups received, respectively, one capsule containing sesame oil (Catalog No. S3547, Sigma-Aldrich, St Louis, MO) or one capsule containing 65 μg of 17β-E (Catalog No. E8875; Sigma-Aldrich) in sesame oil. Each rat in the EP group received two capsules, one containing 65 μg of 17β-E, and the other containing 66 mg of crystalline P (Catalog No. P0130; Sigma-Aldrich). E and P are the major estrogens and progestogens in both rodents and women, and have been used in hormone replacement rodent models (Becker et al., 2005; Frick, 2009). The amounts of E and P were chosen to produce hormone levels that are equivalent to diestrus levels observed in naturally cycling female rats (Dubal and Wise, 2001; Dubal et al., 1998; Mannino et al., 2005). Additionally, we previously showed that these low physiological hormone levels were effective in modulating sleep patterns in young, ovariectomized female rats (Deurveilher et al., 2009, 2011). Given that preparations containing P without E are not commonly used for hormone replacement therapy in women (NAMS, 2012), we did not include animals that only received P. Silastic capsules (1.6 mm inner diameter × 3.2 mm outer diameter; 45 mm in length for oil and E, and 55 mm in length for P; Dow Corning Corporation, Midland, MI) were inserted subcutaneously lateral to the abdominal incision.

All animals, still under anesthesia, were then implanted with electroencephalogram (EEG) and electromyogram (EMG) electrodes for sleep–wake recording. Two miniature screw electrodes were placed over the frontal cortex (1 mm rostral to bregma and 2 mm right to the midline) and the occipital cortex (6 mm caudal to bregma and 2 mm left of the midline) to record the EEG. Two wire electrodes were inserted into the dorsal neck muscles to record the EMG. All electrodes were connected to a small plastic connector and the head cap assembly was secured to the skull using dental acrylic. Following surgery, animals were injected subcutaneously with an analgesic (Ketoprofen, 5 mg/kg) and an antibiotic (Enrofloxacin, 2.5 mg/kg), and monitored for recovery from anesthesia before being returned to the animal colony to be housed singly.

Experimental design

Nine or 10 days after surgery, each rat was transferred to a clear Plexiglas cage (40 × 30 × 40 cm³) placed inside an individual experimental chamber that was equipped with a fan and an incandescent light controlled by a timer to maintain the same 12-h light:12-h dark cycle as in the colony room. On the next day, rats were connected to a flexible cable attached to a rotating commutator, and remained connected for a habituation period of 3–4 days before polygraphic recording started. EEG/EMG recording began at mid-light phase (ZT6) with a baseline 24 h period, followed by sleep deprivation for 6 h over the second half of the light phase (ZT6–12). Sleep deprivation was conducted by introducing novel objects (plastic items such as tubes, cups, and toys of different shapes) into the rats' cages, gently shuffling the bedding, tapping on the side of the cage, and, when necessary, slowly moving the litter tray. These stimuli were applied only when the rats showed behavioral signs of sleepiness (i.e., when they assumed a sleeping posture) or when slow waves were evident in the EEG. After sleep deprivation, the rats were left undisturbed with EEG and EMG recorded for another 24 h