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Sex- and Age-dependent Differences in Sleep-wake Characteristics of Fisher-344 Rats

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Abstract—Aging is a well-recognized risk factor for sleep disruption. The characteristics of sleep in aging include its disruption by frequent awakenings, a decline in both non-rapid eye movement (nonREM) and REM sleep amounts, and a weaker homeostatic response to sleep loss. Evidence also suggests that sleep in females is more sensitive to changes in the ovarian steroidal milieu. The Fischer-344 rats are commonly used experimental subjects in behavioral and physiological studies, including sleep and aging. Most sleep studies in Fischer-344 rats have used male subjects to avoid interactions between the estrus and sleep-waking cycles. The changes in the sleep-wake organization of female Fischer-344 rats, especially with advancing age, are not well-characterized. We determined sleep-waking features of cycling females across estrus stages. We also compared spontaneous and homeostatic sleep response profiles of young (3-4 months) and old (24-25 months) male and female Fischer-344 rats. The results suggest that: i) sleep-wake architectures across stages of estrus cycle in young females were largely comparable except for a significant suppression of REM sleep at proestrus night and an increase in REM sleep the following day; ii) despite hormonal differences, sleep-wake architecture in male and female rats of corresponding ages were comparable except for the suppression of REM sleep at proestrus night and higher nonREM delta power in recovery sleep; and iii) aging significantly affected sleep-wake amounts, sleep-wake stability, and homeostatic response to sleep loss in both male and female rats and that the adverse effects of aging were largely comparable in both sexes. Published by Elsevier Ltd on behalf of IBRO.

Key words: rat, sleep, wakefulness, aging, male, female.

INTRODUCTION

Aging may simply be defined as the time-dependent functional decline that affects most biological systems and living organisms. One of the common features of aging in humans and other mammalian species is its adverse effects on sleep-wake organization. These adverse effects include difficulty falling asleep and sleep disruption by frequent awakenings during night or resting-phase, a decline in both non-rapid eye movement (nonREM) and rapid-eye movement (REM) sleep amounts, a dampening of the sleep/wake circadian rhythm amplitudes, and a decrease in nonREM sleep delta power, an index of prior-time waking or sleep need and the restorative properties of nonREM sleep, during recovery from sleep deprivation or a weaker homeostatic response to sleep loss (Bowersox et al., 1984; Dijk and Duffy, 1999; Shiromani et al., 2000; Panossian et al., 2011; Hasan et al., 2012). Aging is also associated with shortening of waking bouts with sleep intrusions and shortened latencies to sleep during day or active-phase in mammalian species including rodents (Mendelson and Bergmann, 1999; Wimmer et al., 2013; Mander et al., 2017; Kostin et al., 2019).

The findings of studies investigating the effects of sex differences and sex steroids on sleep have been somewhat mixed (Baker and Driver, 2007; Lord et al., 2014; Mong and Cusmano, 2016). However, both human and animal studies suggest that gonadal steroids play a role in sleep modulation and that in females sleep is sensitive to changes in the ovarian steroids (Fang and Fishbein, 1996; Franken et al., 2006; Koehl et al., 2006; Paul et al., 2006; Lord et al., 2014; Mong and Cusmano, 2016). For example sleep complaints in women often

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Abbreviations: AW, active-waking; DE, diestrus; EEG, electroencephalogram; EMG, electromyogram; FFT, Fast Furier Transform; ME, metestrus; nonREM, non-rapid eye movement; QW, quiet-waking; REM, rapid-eye movement.

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coincide with periods of ovarian steroidal fluctuations such as with puberty, menstruation, and menopausal transition. In menstruating women sleep is most disturbed during the luteal phase, when the ovarian steroid levels are elevated (Baker and Driver, 2007; Mong and Cusmano, 2016). Women report considerably more sleep problems than men and are also at significantly higher risk for developing insomnia throughout their lifetime (Lindberg et al., 1997; Ohayon et al., 2004). In rodents, compared to males, cycling females spend less time in sleep, especially REM sleep, on proestrus night, when levels of ovarian steroids, estradiol and progesterone, are elevated (Yamaoka, 1980; Fang and Fishbein, 1996; Franken et al., 2006; Koehl et al., 2006; Mong and Cusmano, 2016). Ovariectomy eliminates this suppression of sleep on the proestrus night and hormone replacement in ovariectomized animals reinstates it (Paul et al., 2006; Deurveilher et al., 2009; Cusmano et al., 2014). Sleep in male rodents seems to be less sensitive to sex steroids as castration does not significantly alter sleep and wakefulness compared to gonadally intact males (Wibowo et al., 2012; Cusmano et al., 2014; Mong and Cusmano, 2016).

The Fischer-344 rats are one of the commonly used strains in studies on various behavioral, physiological, and pathophysiological processes, including sleep and aging (Tani and Ishihara, 1988; Houston et al., 1999; Mendelson and Bergmann, 1999; Desarnaud et al., 2004; Blanco-Centurion and Shiromani, 2006). In these rats estrus cycles start approximately at the age of 1.5 months and continue steadily until 17 months of age (Sone et al., 2007). On average each cycle lasts for 5 days and broadly consists of four stages, namely, proestrus, estrus, metestrus (ME), and diestrus (DE). These stage are accompanied by certain changes in reproductive organs and hypothalamic-pituitary-ovarian reproductive axis that result into behavioral changes.

Although, biological sex and aging have been recognized by the National Institutes of Health as critical risk factors for sleep disruption or insomnia (2005), most rodent sleep studies including those in Fischer-344 rats have used mainly male subjects to avoid confounds of hormonal variation during the estrus cycle in females. A better understanding of sleep-wake characteristics of Fischer-344 female rats, especially with advancing age is needed.

In this study we systematically characterized sleep-wake parameters in young (3–4 months old) cycling Fischer-344 females rats across different phases of estrus cycle. We further compared spontaneous and homeostatic sleep response profiles of young male and female and old (24–25 months old) male and female Fischer-344 rats. The results suggest: a) that sleep-wake architecture was comparable across the estrus cycle in female rats except for a significant decline in REM sleep at proestrus night and an increase in REM sleep the following day; b) despite hormonal differences, sleep-wake architecture in male and female Fischer-344 rats of corresponding ages did not differ except for the suppression of REM sleep at proestrus night and higher nonREM delta power in recovery sleep following sleep deprivation in females; and c) that aging significantly affected the sleep-wake organization in both male and female rats and the adverse effects of aging were comparable in both sexes.

EXPERIMENTAL PROCEDURES

Experimental subjects

Experiments were conducted on male and female Fischer 344 rats that were 3–4 month old (young) and 24–25 month old (old) at the time of data acquisition. A total of 11 young male, 10 young female, 12 old male, and 13 old female rats were used. One young male, one young female, 2 old males and one old female could not be studied due to poor recording quality or poor health. Rats were maintained on a 12:12 light: dark cycle (lights on at 8:00 h, illumination intensity about 100 lux), and an ambient temperature of 24 ± 2 °C. Food and water were available *ad libitum* throughout the studies. All experiments were conducted in accordance with the National Research Council's "Guide for the Care and Use of Laboratory Animals" and were approved by the Institutional Animal Research Committee of the Veterans Affairs Greater Los Angeles Healthcare System.

Surgical procedures

The details of the surgical procedures have been described previously (Alam and Mallick, 2008; Kostin et al., 2013). Briefly, under anesthesia (Ketamine + Xylazine: 80:10 mg/kg; i.p.) and aseptic conditions, electroencephalogram (EEG) and electromyogram (EMG) electrodes were implanted for polygraphic monitoring of sleep-waking states. Electrodes were soldered to a miniature plug, which was anchored to the skull with dental acrylic.

Recovery and adaptation

After surgery, rats were placed in their Plexiglas cages, placed in a sound attenuated and temperature controlled recording chamber and were allowed to recover from the surgical procedure for at least 10 days. Older rats were given at least 14 days for recovery. The rats were then connected to the recording cables and allowed an additional seven days for acclimatization with the recording setup and recording cables before experiments were begun. Both young and old male or female rats were housed in the same recording chamber and their sleep-wake parameters studied together.

Vaginal smears and assessment of estrous cycle in young female rats

Stages of estrous cycle were determined by cytological analysis of vaginal smears, which were taken only from young female rats, since occurrence of cycle ceases by 16–17 months of age (Sone et al., 2007). The estrous cycle in young Fischer-344 rats typically lasts for 4–6 days (Sone et al., 2007). We collected smears every day for 12 days to include 2–3 cycles. During those

12 days, EEG and EMG were continuously recorded. Smears were collected 10–15 minutes before the onset of dark or active period so that the procedure had minimal influence on sleep-wake cycle. Collection of one smear took about 1 min. The technique of sample collection was adopted from other studies with some modifications (Marcondes et al., 2002; Goldman et al., 2007; Paccola et al., 2013). Briefly, the rat was gently taken out of the cage without disconnecting the recording cable. The upper part of body was wrapped in a towel and the rat was turned to an up-side-down position. At this point 15 μl of sterile saline was injected into vagina and sucked back at least 3 times by micropipette. Saline drops with the vaginal smear were transferred to the microscope glass slides, cover-slipped, and assessed under light microscope immediately after sample collection.

The phases of the estrus cycle were determined by the proportion of leukocytes, nucleated epithelial cells, and anucleated cornified cells in the vaginal smear using standard criteria (Marcondes et al., 2002; Goldman et al., 2007; Paccola et al., 2013). A proestrus smear predominantly consisted of nucleated epithelial cells; an estrous smear predominantly consisted of anucleated cornified cells; a ME smear consisted of same proportion of leukocytes, cornified cells, and nucleated epithelial cells; and a DE smear predominantly consisted of leukocytes (Marcondes et al., 2002). The length of the cycle was determined as the number of consecutive days from the day of the appearance of the cornified cells in the smear to the day prior to the next cornified cell phase.

Data acquisition

After adaptation, rats were undisturbed, except for 10–15 min of routine checkup/maintenance each day and for taking smears. Amplified and filtered EEG and EMG signals were continuously recorded and stored on the hard-disc of the computer, using an integrated computer interface device (Cambridge Electronic Design 1401 with Spike 2 software; London) for subsequent sleep-wake scoring and analyses. We scored sleep-wake data from the second estrus cycle, so the rats were further acclimated to the smear collection procedure.

After spontaneous sleep-wake recordings, rats were subjected to 6 h of total sleep deprivation stating at light-onset. Rats were sleep-deprived using a gentle handling and environmental enriching procedure, which included introduction of new objects into the cage in order to keep the animals occupied and replacing them with new ones when animals appeared to become drowsy, as described previously (Kostin et al., 2013; Alam et al., 2014). Following sleep-deprivation rats were left undisturbed and their sleep-wake profile recorded for another 4–6 h. Previous studies indicate that the homeostatic response to sleep loss were comparable in proestrus and estrus phases (Schwierin et al., 1998) and that sleep-wake architecture during the light-phase are largely comparable in cycling females. Therefore, sleep deprivation studies were conducted without regard to estrous phase in young female rats.

Data analyses

Sleep-wake profiles were scored manually in 10 s epochs for active-waking (AW), quiet-waking (QW), nonREM sleep, and REM sleep using SleepSign software and standard criteria (Alam and Mallick, 2008; Kostin et al., 2013). The parameters analyzed included time spent in each state and the duration and frequency of each state. The continuity of each sleep-wake state was determined by classifying each episode of the 4 sleep-wake stages, based on their duration, into 5 sub-categories: bouts with duration of <30 s (very short), >30 s to 2 min (short), >2 min to 5 min (medium), >5 min to 10 min (long), and >10 min (extra-long).

Earlier studies in rats and mice indicate that highest variability in sleep characteristics occur during the night of the proestrus phase and the day before or next to it while sleep-waking on other days of the estrus cycle including ME and DE did not show significant variations. Therefore, for sleep-wake scoring and analysis in young female rats, three consecutive 24 h recordings that included 24 h before proestrus (ME-DE combined), 24 h of proestrus, and 24 h after proestrus (estrus) were selected. For young male as well as old male and female rats, data from 2 consecutive days were analyzed and averaged for analysis to minimize variability.

Delta spectral analysis was performed using Fast Furier Transform (FFT; 0.7–4.0 Hz). Given that homeostatic recovery is most robust during first 3 h (Huber et al., 2000) and to minimize circadian effects on sleep-wake architecture, we used first 3 h of recovery for analysis of homeostatic sleep response to 6 h of sleep deprivation. Delta power in nonREM sleep during the recovery period was calculated on an hourly basis and pooled for the first 3 h for statistical comparison. The data presented are percentage change compared to the delta power in baseline nonREM sleep during comparable time period (ZT 7–10) in undisturbed rats taken as 100%.

The SigmaPlot (Systat Software, San Jose, USA) software package was used for statistical analyses of the data. One Way Repeated Measures Analysis of Variance (RM ANOVA) followed by pair-wise multiple comparisons using Holm-Sidak test was used for comparing sleep-wake amounts and sleep-wake stability (frequency of AW, QW, nonREM sleep, and REM sleep episodes of various bout lengths) during the dark- or the light-phase across different stages of the estrus cycle in young females. The spontaneous sleep-wake amounts and sleep-wake stability of young female during estrus phase, young male, old male, and old female rats during the dark-phase or light-phase as well as their homeostatic response to sleep deprivation were compared using Two-Way ANOVA with sex and age as factors and their interactions within each time of the day/night followed by multiple comparisons using the

Holm-Sidak test, when the interaction term was significant. Percent change in delta power during recovery sleep between groups were compared using un-paired t-test. In some cases, the data failed normality test but passed equal variance test. Those data points were analyzed by both nonparametric and parametric tests and a more conservative of the two values, which mostly was with parametric test, was used as level of significance.

RESULTS

A. Sleep-wake organization in young female Fischer-344 rats across estrus cycle

The sleep-wake profiles of 9 young female rats were analyzed across their estrus cycle. Cytological analysis of smear revealed that 6 of these 9 rats had estrus cycles lasting for 5 days. Those rats showed 3 cornified cell phases during 12 days of sample collection period, i.e., two full estrus cycle (Fig. 1). The remaining 3 rats showed at least two cornified cell phases during the 12 days. We compared sleep-wake profiles of cycling females during proestrus, estrus, and combined ME-DE phases.

Sleep-wake amount. The 24 h sleep-wake amounts of young cycling female across stages of estrus cycle are shown in Fig. 2. During the dark (active) phase there was a significant effect of estrus cycle on the amounts of AW (One-Way RM ANOVA, $F_{2,8} = 6.17$, $p = 0.010$), nonREM sleep ($F_{2,8} = 4.49$, $p = 0.028$), and REM sleep ($F_{2,8} = 21.56$, $p = 0.001$). All pairwise multiple comparison using Holm-Sidak test revealed that the cycling females spent significantly more time in AW ($p < 0.05$; Fig. 2, A,B) and less time in nonREM sleep ($p < 0.01$; Fig. 2, E,F) and REM sleep ($p < 0.01$; Fig. 2, G,H) during the proestrus night compared to estrus night. Compared to ME-DE, while proestrus females exhibited comparable amounts of AW ($p = 0.07$) and nonREM sleep ($p = 0.10$), their REM sleep amount remained significantly lower ($p < 0.01$). The decline in REM sleep amount during proestrus phase compared to estrus and ME-DE phases was much stronger, compared to changes in AW or nonREM sleep and persisted throughout the night (Fig. 2, G). The decrease in REM sleep amount at proestrus night was also significantly lower compared to REM sleep amount during the dark-phase in young male rats ($p < 0.01$). QW amounts were comparable cross cycling stages.

During the light-phase or the day following the proestrus night, AW (One-Way RM ANOVA, $F_{2,8} = 1.08$, $p = 0.346$), QW ($F_{2,8} = 3.41$, $p = 0.059$), and nonREM sleep ($F_{2,8} = 1.43$, $p = 0.268$), amounts in cycling females were comparable to ME-DE, and estrus phases, except that the proestrus females spent significantly more time in REM sleep ($F_{2,8} = 7.18$, $p = 0.006$) compared to ME-DE ($p < 0.01$; Holm-Sidak test), especially during the early light phase (Fig. 2, G,H).

Sleep-wake stability. In order to characterize the effect of estrus cycle on sleep–wake stability, we sub-grouped each AW, QW, nonREM sleep, and REM sleep episodes into very short ($\leq$30 s), short (> 30 s – 2 min), medium (> 2 min – 5 min), long (> 5 min – 10 min) and extra-long (> 10 min) bout lengths and compared their frequencies across stages of the estrus cycle. Fig. 3 shows a comparison of the frequencies of AW, QW, nonREM sleep, and REM sleep episodes of various bout lengths encountered in young cycling female rats.

During the dark phase, the frequencies of AW, QW, nonREM sleep, and REM sleep episodes of various durations were largely comparable in cycling females except that: i) proestrus females exhibited higher number of short-duration AW bouts compared to estrus and ME-DE phases (One-Way RM ANOVA, $F_{2,8} = 4.33$, $p = 0.031$; Fig. 3, A); ii) proestrus and estrus females also exhibited higher number of AW bouts of longer duration compared to DE-ME and estrus phases ($F_{2,8} = 10.41$, $p = 0.001$; Fig. 3, A); iii) proestrus females exhibited significantly fewer episodes of medium ($F_{2,8} = 10.01$, $p = 0.002$) and longer bouts of nonREM sleep ($F_{2,8} = 5.43$, $p = 0.016$; Fig. 3, C) compared to both estrus and ME-DE phases; and iv) proestrus females exhibited a significantly decreased number of REM sleep episodes of all bout lengths encountered compared to both estrus and ME-DE phases ($F_{2,8} = 7.93$–20.69, $p = 0.004$–$0 < 0.001$; Fig. 3, D).

During the light phase, the frequencies of AW, QW, nonREM

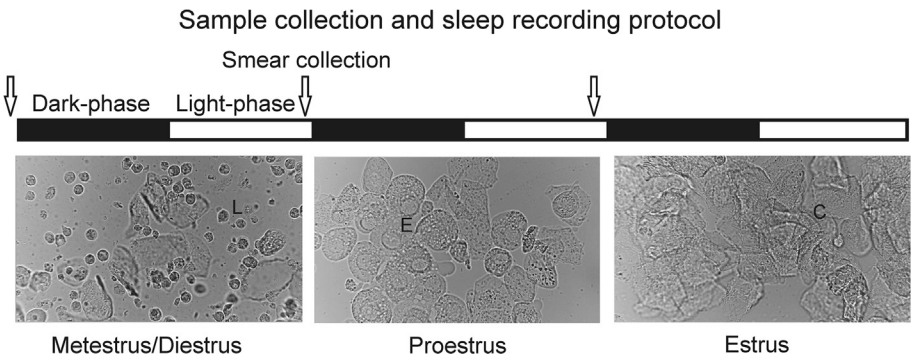


Fig. 1. Experimental design and identification of estrus phases. Photomicrographs of unstained vaginal smear from female rats representing metestrus/diestrus, proestrus, and estrus phases of the estrus cycle. The proportion of epithelial cells (E, round and nucleated), cornified cells (C, anucleated and irregular), and leukocytes (L, small and round) in vaginal smear was used for determining phases of estrus cycle. Please note a much higher proportion of leukocytes as well as anucleated cornfield cells in ME phase, diestrus phase consisted predominantly of leukocytes, a predominance of nucleated epithelial cell in proestrus phase, and that of cornified cell in the estrus phase. We compared sleep-wake profiles in cycling females across 72 h, which included 24 h prior to proestrus (and typically consisted of ME or ME-DE transition (ME-DE), 24 h of proestrus, and 24 h after proestrus (estrus). The smear was collected 15 min before the dark-onset.

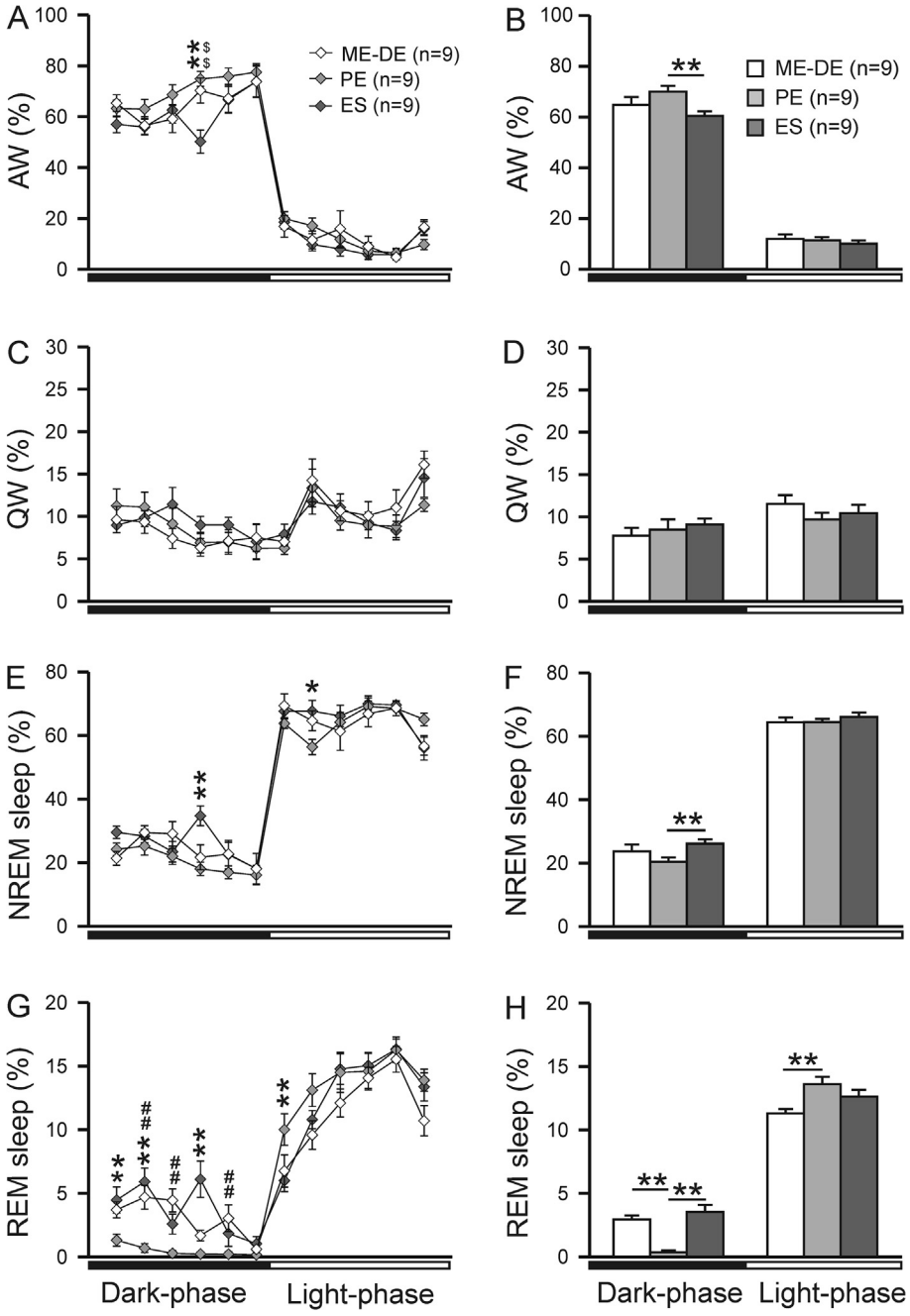


Fig. 2. Sleep-wake amount across estrus cycle in Fischer-344 rats. Percent time (mean ± SEM) spent by young cycling females (n = 9) in AW, QW, nonREM sleep, and REM sleep at every 2 h interval (left side, **A, C, E, G**) and during the 12 h each of the dark and light cycle (right-side, **B, D, F, H**) during ME-DE, proestrus, and estrus phases. Dark phase is marked by dark line. Proestrus females spent significantly more time in AW, and less time in nonREM and REM sleep during the dark-phase and more time in REM sleep during the light-phase or the following day. ME-DE, metestrus-diestrus; PE, proestrus, ES, estrus; *, ES vs. PE stage or between marked bars (on the right); #, ME-DE vs. PE stage; $$, ES versus ME-DE. *, $, # = $p < 0.05$; **, $$, ##, = $p < 0.01$ (One-Way RM ANOVA followed by Holm-Sidak test).

sleep, and REM sleep episodes of different bout durations were also comparable in cycling females except that: i) both proestrus and estrus females exhibited significantly fewer episodes of QW (< 30 s duration) compared to ME-DE phase (One-Way RM ANOVA, $F_{2,8} = 4.235$, $p = 0.033$; Fig. 3, B); and ii) proestrus females exhibited higher number of REM sleep episodes of > 30 s – 2 min duration compared to ME-DE and estrus phases ($F_{2,8} = 4.72$, $p = 0.025$; Fig. 3, D).

B. Sleep-wake organization in young versus old female and male rats

We compared sleep-wake profiles of young male (n = 10), young female (n = 9), old male (n = 10) and old female (n = 12) rats to determine age-dependent changes in sleep-wake architecture in both sexes. Since cycling females exhibited comparable sleep-wake profiles in estrus and ME-DE phases (Figs. 2 and 3) that together constitute > 80% of the estrus cycle, we chose to compare sleep-wake parameters in estrus female as a representative of sleep-wake profiles in young females.

Sleep-wake amounts: effects of age. The amounts of AW, QW, nonREM sleep, and REM sleep across the light–dark cycle in young and old rats of both sexes are shown in Fig. 4. During the dark phase, aging significantly affected amounts of AW (Two Way ANOVA, $F_{1,37} = 39.19$, $p \leq 0.001$; Fig. 4, A,B), QW ($F_{1,37} = 27.61$, $p \leq 0.001$; Fig. 4, C,D), nonREM sleep ($F_{1,37} = 17.64$, $p \leq 0.001$; Fig. 4, E,F), and REM sleep ($F_{1,37} = 13.67$, $p \leq 0.001$; Fig. 4, G,H) in both male and female rats. The effects of aging were also observed on the amounts of AW (Two Way ANOVA, $F_{1,37} = 7.74$, $p = 0.008$), QW ($F_{1,37} = 32.20$, $p \leq 0.001$), nonREM sleep ($F_{1,37} = 31.83$, $p \leq 0.001$), and REM sleep ($F_{1,37} = 24.43$, $p \leq 0.001$) in both male and female rats during the light phase (Fig. 4, A–H).

Young female versus old female. During the dark phase, compared to young, old female rats spent significantly less time in AW ($p < 0.01$; Holm-Sidak test; Fig. 4, A,B) and more time in QW ($p < 0.01$; Fig. 4, C,D). These changes were much evident during the last part of the dark-phase

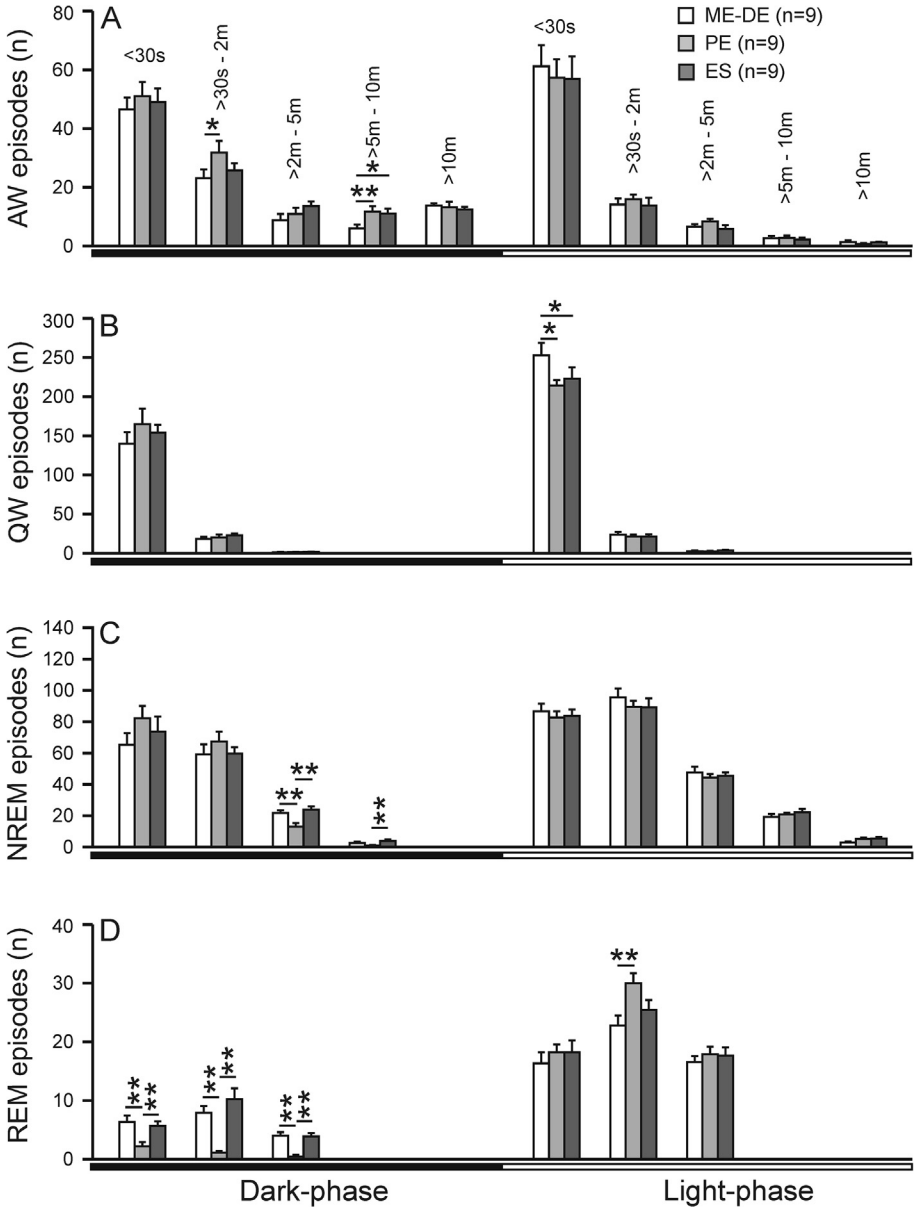


Fig. 3. Sleep-wake stability across estrus cycle in Fischer-344 rats. The number (mean ± SEM) of AW (**A**), QW (**B**), nonREM sleep (**C**), and REM sleep (**D**) episodes per 12 h during the dark-phase (marked by dark line) and light-phase in young cycling females during ME-DE, proestrus, and estrus phases. Overall, proestrus female exhibited significant suppression of REM sleep episodes of all bout lengths, and nonREM sleep episodes of medium and long bout durations, while AW episodes were much frequent during the dark-period compared to other phases of the estrus cycle. During the light-period, however, there was a significant increase in > 30 s – 2 min bouts of REM sleep. Except for the REM sleep the frequency of various sleep-wake episodes were largely comparable. *, $p < 0.05$; **, $p < 0.01$ (One Way RM ANOVA followed by Holm-Sidak test).

(Fig. 4, A–D). Old females also exhibited significant increases in the amounts of nonREM ($p < 0.05$; Fig. 4, E,F) and REM sleep ($p < 0.01$; Fig. 4, G,H), especially during the last part of dark-period.

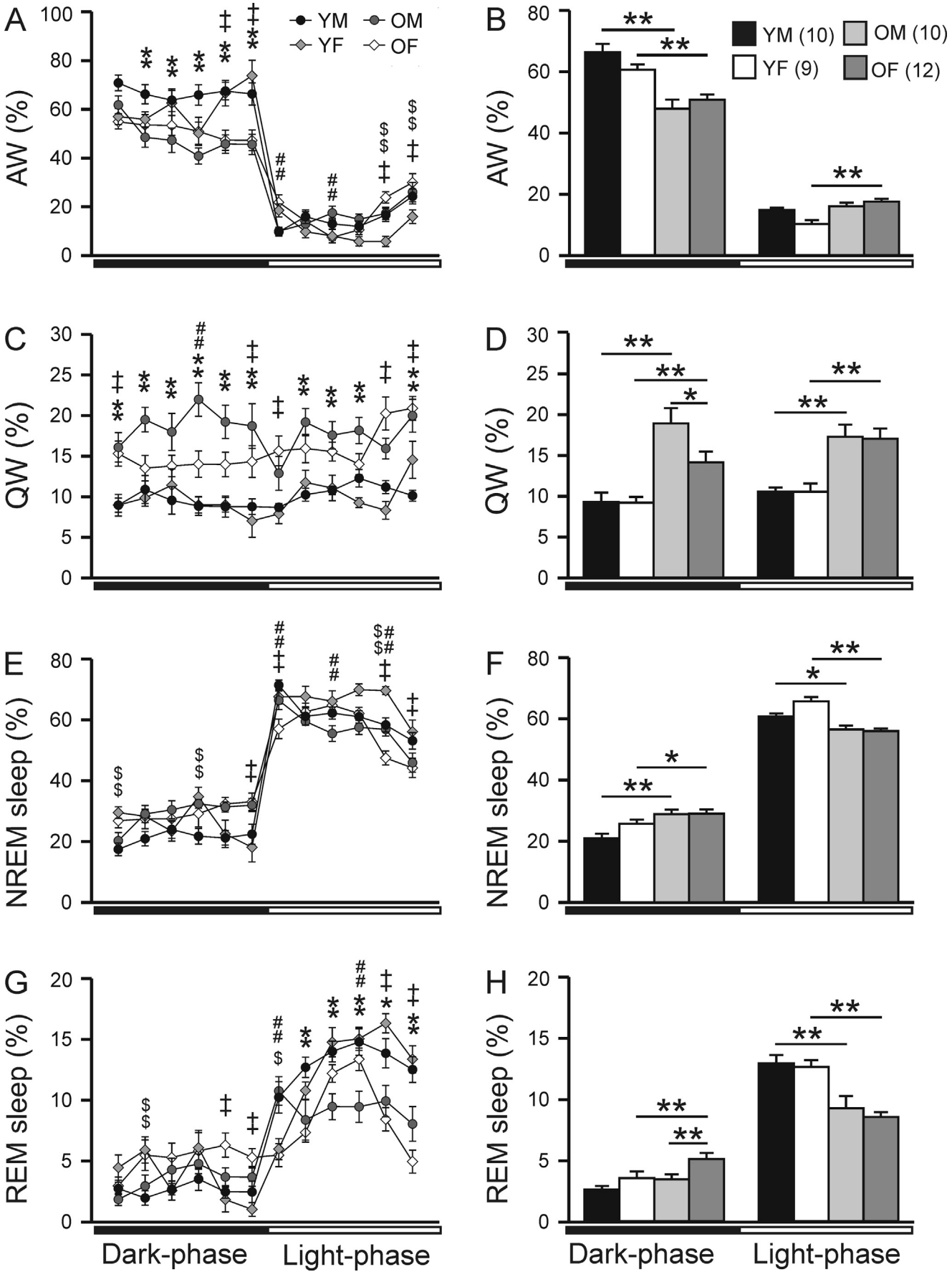
During the light phase, compared to young, old female rats spent significantly more time in AW ($p < 0.01$, Holm-Sidak test) and QW ($p < 0.01$), especially during the latter part of the light-period (Fig. 4, A–D). Old female rats also spent significant less time in nonREM sleep ($p < 0.01$) and REM sleep ($p < 0.01$), especially during the latter part of the light-period. (Fig. 4, E–H).

Young male versus old male. During the dark phase, compared to young, old male rats also spent significantly less time in AW ($p < 0.01$, Holm-Sidak test; Fig. 4, A,B) and significantly more time in QW ($p < 0.01$; Fig. 4, C, D). These changes were significant across the dark-period (Fig. 4, A–D). Old male rats also exhibited significantly increased amount of nonREM sleep ($p < 0.01$), although their REM sleep amount were comparable ($p = 0.134$; Fig. 4 E–H).

During the light phase, compared to young, old male rats exhibited comparable changes in AW amount (Fig. 4, A,B), but significantly higher amounts of QW ($p < 0.01$; Fig. 4, C,D). Old male rats also exhibited significant decreases in the amounts of nonREM sleep ($p < 0.05$; Fig. 4, E,F) and REM sleep ($p < 0.01$; Fig. 4, G,H). The decrease in REM sleep amount persisted throughout the light-period (Fig. 4, G).

Sleep-wake amounts: effects of sex. During the dark phase, while there was no significant effect of sex on the amounts of AW (Two Way ANOVA, $F_{1,37} = 0.09$; $p = 0.768$; Fig. 4, A,B) and nonREM sleep ($F_{1,37} = 0.63$, $p = 0.432$; Fig. 4, E,F), it significantly affected amounts of QW ($F_{1,37} = 4.86$; $p = 0.034$; Fig. 4, C,D) and REM sleep ($F_{1,37} = 6.32$, $p \leq 0.016$; Fig. 4, G,H). During the light phase, however, no sex-dependent changes were observed in the amounts of AW ($F_{1,37} = 26$; $p = 0.612$; Fig. 4, A,B), QW ($F_{1,37} = 0.13$; $p = 0.718$; Fig. 4, C,D), nonREM sleep ($F_{1,37} = 1.51$, $p = 0.227$; Fig. 4, E, F) and REM sleep ($F_{1,37} = 3.13$, $p \leq 0.085$; Fig. 4, G,H).

Young female versus young male. Compared to young male, young female rats exhibited comparable amounts of AW, QW, nonREM sleep, and REM sleep during both dark- and light-phase. (Fig. 4, A–H).


A
B
C
D
E
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AW (%)
QW (%)
NREM sleep (%)
REM sleep (%)
YM
OM
YF
OF
YM (10)
OM (10)
YF (9)
OF (12)
Dark-phase
Light-phase

Old female versus old male. During the dark phase, compared to old male, old female rats exhibited comparable amounts of AW and nonREM sleep, but a decreased amount of QW ($p < 0.05$, Holm Sidak test) and increased amount of REM sleep ($p < 0.01$, Holm Sidak test; Fig. 4, A–H). During the light phase, however, old male and female rats exhibited comparable amounts of AW, QW, nonREM sleep, and REM sleep (Fig. 4, A–H)

Sleep-wake stability: effects of age. The frequency of AW, QW, nonREM sleep, and REM sleep episodes of various bout durations that were encountered across the light: dark cycle in young and old rats of both sexes are shown in Fig. 5. Aging significantly and differentially affected the frequency and duration of AW, QW, nonREM sleep and REM sleep episodes across the light: dark cycle (Two-Way ANOVA followed by Holm-Sidak analyses).

Young female versus old female. During the dark phase, compared to young female, old female rats exhibited significantly increased waking instability as marked by: i) significantly increased number of < 30 s ($p < 0.05$, Holm-Sidak test) and > 30 s–2 min ($p < 0.05$) of AW and a decreased number of > 10 min bouts of AW ($p < 0.01$; Fig. 5, A); and ii) significantly increased number of QW episodes of > 30 s ($p < 0.01$; Fig. 5, B). Compared to young female, old female rats also exhibited significantly increased sleep intrusion as marked by: i) significantly increased number of nonREM sleep episodes of < 30 s ($p < 0.05$; Fig. 5, C); and ii) significantly increased number of REM sleep episodes of < 30 s ($p < 0.05$) and > 30 s – 2 min bout lengths ($p < 0.01$; Fig. 5, D).

During the light phase, compared to young female, old female rats exhibited significantly increased sleep instability and waking intrusions as marked by: i) significant increases in the number of AW bouts of > 30 s – 2 min duration ($p < 0.01$; Fig. 5, A); and < 30 s ($p < 0.05$) as well as > 30 s – 2 min episodes of QW ($p < 0.01$; Fig. 5, B); ii) significantly increased number of nonREM sleep episodes of < 30 s ($p < 0.01$) and decreased number of > 5 min–10 min ($p < 0.01$) and > 10 min episodes of nonREM sleep ($p < 0.01$; Fig. 5, C); and iii) significantly decreased number of REM sleep episodes of > 2 min–5 min ($p < 0.01$; Fig. 5, D).

Young male versus old male. During the dark phase, compared to young male, old male rats also exhibited significantly increased waking instability (Fig. 5, A–D) as marked by: i) significantly increased number of AW episodes of > 30 s–2 min ($p < 0.01$) and > 2 min–5 min ($p < 0.01$) and decreased number of AW episodes of > 10 min bout lengths ($p < 0.01$); and ii) significantly increased number of QW episodes of < 30 s ($p < 0.01$), > 30 s–2 min ($p < 0.01$) and > 2 min–5 min ($p < 0.01$). Compared to young male, in old male rats sleep intrusions were also significantly higher as marked by: i) significantly increased numbers of nonREM sleep episodes of < 30 s ($p < 0.01$), > 30 s – 2 min ($p < 0.01$), and > 2 min–5 min ($p < 0.05$); and ii) a significantly increased number of REM sleep episodes of < 30 s ($p < 0.01$) and > 30 s–2 min bout lengths ($p < 0.01$).

During the light phase, compared to young male, old male rats also exhibited increased waking intrusions during sleep and sleep instability (Fig. 5, A–D) as marked by: i) significant increases in the number of AW episodes of > 30 s-2 min ($p < 0.05$) and > 2 min–5 min bout length ($p < 0.05$); ii) significant increases in the number of < 30 s ($p < 0.05$) and > 30 s–2 min episodes of QW ($p < 0.01$); iii) significantly increased number of nonREM sleep episodes of < 30 s ($p < 0.01$) and decreased number of episodes of > 5 min–10 min bout lengths ($p < 0.05$); and iv) significantly decreased number of REM sleep episodes of > 2 min–5 min bout length ($p < 0.01$).

Sleep-wake stability: effects of sex. Young female versus young male. During the dark phase, compared to young male, young female rats exhibited comparable level of sleep-wake stability as marked by number of AW, QW, nonREM sleep, and REM sleep episodes of various duration, except for an increase in the number of > 2 min–5 min episodes of nonREM sleep (Fig. 5, A–D). During the light phase as well, young male and female rats exhibited similar level of sleep-wake stability as the number of AW, QW, nonREM sleep, and REM sleep episodes of various duration did not differ significantly, except for an increase number of longer bouts of nonREM sleep in females ($p < 0.05$; Fig. 5, A–D)

Old female versus old male. During the dark-phase, compared old male, old female rats exhibited comparable levels of waking instability as well as sleep intrusions, as marked by insignificant differences in the number of AW, QW, nonREM sleep, and REM sleep episodes of various duration except for, i) decreased number of short ($p < 0.05$) and medium ($p < 0.05$) bouts of QW; and ii) increased number of medium bouts of REM sleep ($p < 0.05$; Fig. 5, A–D). During day, both old male and female rats exhibited comparable and

Fig. 4. Effects of aging on sleep-wake amounts in male and female Fischer-344 rats. Percent time (mean ± SEM) spent by young and old male versus female Fischer-344 rats in AW, QW, nonREM sleep, and REM sleep at every 2 h interval (left side, **A, C, E, G**) and during the 12 h each of the dark and light cycle (right-side, **B, D, F, H**). Dark phase is marked by dark line. Both old male and female rats exhibited a decline in AW amounts, an increase in QW and nonREM sleep amounts during the dark-phase and suppression of nonREM and REM sleep and an increase in waking during the light-phase. The sleep-wake amounts in young and old male compared to young and old female rats, respectively, were comparable. YM, young male; YF, young female (estrus), OM, old male; OF, old female; *, young male vs. old male or between the marked bars; +, young female vs. old female; $, young male vs. young female; #, old male vs. old female. *, +, $, #, = $p < 0.05$; **, ++, $$, ##, = $p < 0.01$ level of significance (Two Way ANOVA followed by Holm-Sidak test).

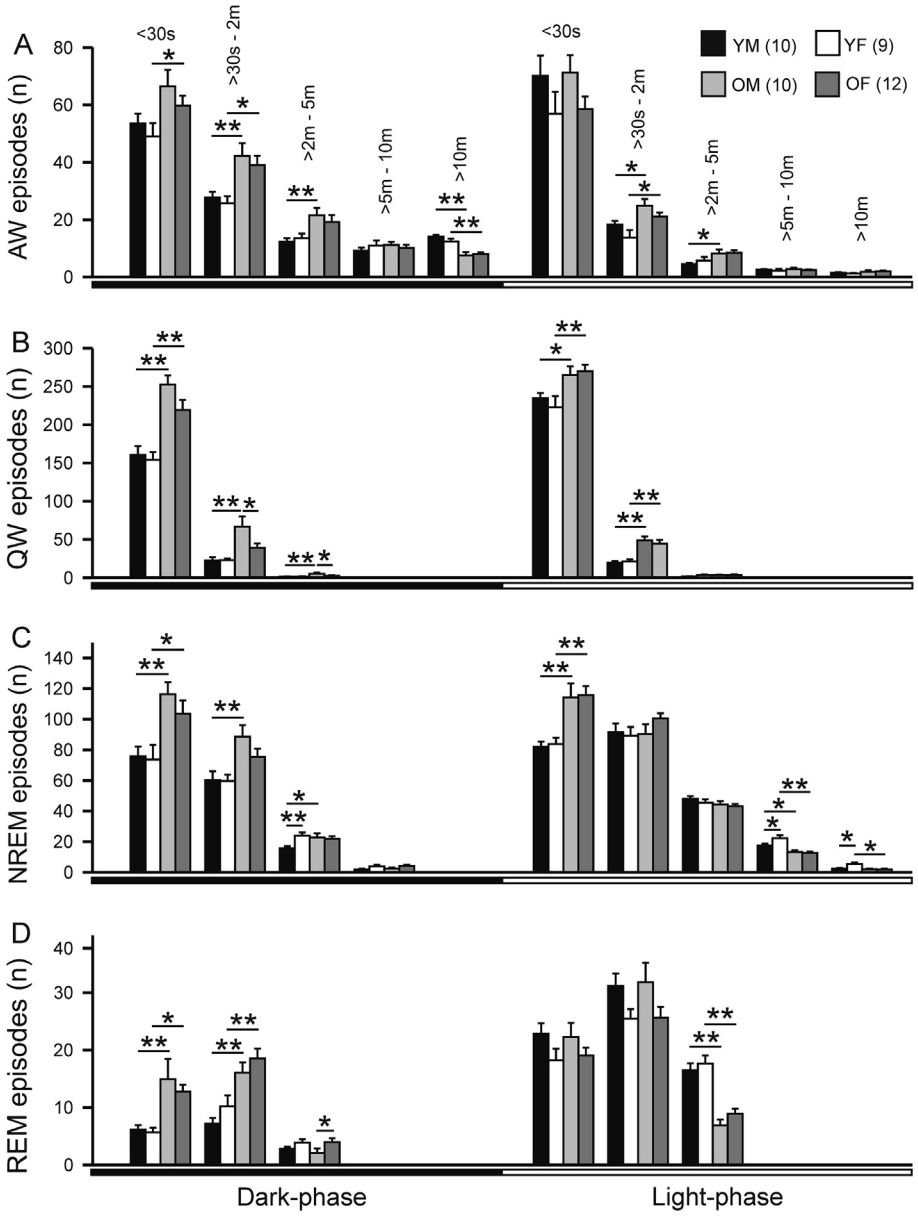

Fig. 5. Effects of aging on sleep-wake stability in male and female Fischer-344 rats. The number (mean ± SEM) of AW (**A**), QW (**B**), nonREM sleep (**C**), and REM sleep (**D**) episodes per 12 h during the dark-phase (marked by dark line) and light phase in young male and female and old male and female rats. Overall, both old male and female rats exhibited significant waking instability as marked by increases in short duration and decreases in long duration bouts of AW and sleep intrusion as marked by increased frequency of nonREM and REM sleep episodes during the dark-phase. On the contrary, during the light-phase, both old male and female rats exhibited sleep disruption as marked by increased number of short duration and decreased number of long and extra-long bouts of nonREM and REM sleep; and waking intrusions as marked by increased frequency of AW and QW bouts. However, these adverse effects of aging were largely comparable in both young and old male and female rats. *, $p < 0.05$; **, $p < 0.01$ (Two Way ANOVA followed by Holm-Sidak test).

significantly increased levels of waking intrusions and sleep instability as changes in number of AW, QW, nonREM sleep, and REM sleep episodes in these rats did not differ significantly (Fig. 5, A–D).

Homeostatic sleep response. We compared sleep–wake architecture of young and old male and female rats during the first 3 h of recovery period after 6 h of sleep deprivation to determine if young and old female rats exhibit any difference in their homeostatic sleep response compared to their male counterparts (Table 1). Both young and old male and female rats exhibited decreased waking and increased sleep during the recovery period following sleep deprivation (Two-Way ANOVA followed by Holm-Sidak test). However, the following age- and sex-related differences were observed.

Young female versus old female. Compared to young female, old female rats exhibited a muted response to sleep deprivation. During recovery period, while the amounts of AW, QW, and nonREM sleep were comparable, old rats exhibited a decreased REM sleep rebound ($p < 0.01$) and a decreased EEG delta activity in nonREM rebound sleep [180 ± 8% of the baseline nonREM sleep delta ($n = 9$) versus 155 ± 4 of the baseline nonREM sleep delta ($n = 9$), $t = 2.766$, $p < 0.01$].

Young male versus old male. During recovery period, compared to young male, old male rats exhibited: i) comparable amount of AW and QW; ii) While the amount of nonREM sleep was comparable, EEG delta activity in nonREM sleep was significantly lower [154 ± 3% of the baseline nonREM sleep delta ($n = 10$) versus 141 ± 4% of the baseline nonREM sleep delta ($n = 10$), $t = 2.571$, $p < 0.05$]; and iii) a decreased REM sleep rebound ($p < 0.01$).

Young female versus young male. During 3 h of recovery period, compared to young male, young female rats exhibited: i) comparable amounts of AW, QW and REM sleep; ii) a greater nonREM sleep rebound ($p < 0.05$); and iii) increased EEG delta activity in nonREM sleep [154 ± 3% versus 180 ± 8%, $t = 3.078$, $p < 0.01$].

Old female versus old male. Both old male and female rats exhibited comparable amounts of AW, QW, nonREM sleep, and REM sleep during recovery period. However,

Table 1. S-W organization during 3 h of baseline and 3 h of recovery period after 6 h of sleep deprivation

Sleep-waking amounts (%, mean ± SEM)								
S-W state	Young male (n = 10)		Young female (n = 9)		Old male (n = 10)		Old female (n = 12)	
	Baseline	Recovery	Baseline	Recovery	Baseline	Recovery	Baseline	Recovery
AW	17.2 ± 1.6	6.1 ± 0.8 $$	6.7 ± 0.8 #	2.2 ± 0.5 $$	13.2 ± 1.8	7.4 ± 1.4 $	10.7 ± 2.2	4.7 ± 1.5 $
QW	12.2 ± 1.6	5.7 ± 0.7 $$	13.9 ± 1.9	4.0 ± 0.5 $$	16.6 ± 1.1 # *	10.6 ± 2.2 $$	11.8 ± 1.0	8.3 ± 1.4 $
NREM sleep	56.9 ± 1.9	67.8 ± 1.2 $$	62.5 ± 1.5 #	75.6 ± 1.3 # $$	59.7 ± 1.7	69.5 ± 1.9 $$	64.1 ± 2.0	75.4 ± 1.9 $$
REM sleep	13.5 ± 0.8	20.4 ± 0.9 $$	16.7 ± 0.9	18.3 ± 0.9	10.3 ± 1.1	12.5 ± 1.3 **	12.8 ± 1.3	11.5 ± 1.3 **

*, Young versus Old; #, Male versus Female; $, baseline versus recovery (Two-Way ANOVA followed by Holm-Sidak test). *, #, $ = < 0.05; **, ##, $$ = $p < 0.01$.

EEG delta activity in nonREM sleep was significantly higher in old female rats compared to its male counterpart (155 ± 4% versus 141 ± 4%, $t = 2.30$, $p < 0.05$).

DISCUSSION

In this study we characterized sex- and age-related changes in the characteristics of spontaneous sleep-waking as well as homeostatic responses to sleep deprivation in Fischer-344 rats, a commonly used strain in studies on various behavioral and pathophysiological processes, including sleep and aging. Although, biological sex and aging are two well-recognized risk factors for sleep disruption, most sleep studies in Fischer-344 rats have used only male subjects, and aging and sex-dependent changes in sleep-wake architecture in Fischer-344 rats are not fully described. The young and old rats used in this study were 3–4 months old and 24–25 months old, respectively. It is difficult to equate rat data to age equivalents in humans. However, according to one estimate, the age of young rats used in this study roughly correspond to 21–26 years of sexually mature and reproductive adult and that of old rats correspond to 61–66 years old human, an age group when both women and Fischer-344 rats exhibit reproductive senescence (Quinn, 2005; Sone et al., 2007). However, unlike females, male rats are fertile at even 500 days of age and reproductive senescence in males appears to be highly variable and even possibly non-existent in some extremely old animals and men (Saksena et al., 1979).

The reproductive life span of female Fischer-344 rats broadly consists of the onset of estrus cycle at about 1.5 months, the recurrence of a regular cornified cell phase for up to 9 months, and then at irregular or extended intervals and finally cessation of the cycle at about 17 months of age. The cessation of estrus cycle is followed by irregular appearance of single cornified cell phase or repetitive pseudopregnancy phase, which disappears at 27 months and is followed by persistent anestrus state (Sone et al., 2007). Thus the young and old female Fischer-344 rats used in this study were in their early and almost at the end of the reproductive period, respectively.

Sleep-wake organization in young cycling females

We found that the young cycling females, except for the proestrus phase, exhibit similar sleep-wake organization as the amounts of AW, QW, nonREM sleep, and REM sleep and the stability of those states as indicated by number and duration of AW, QW, nonREM and REM sleep episodes were fundamentally unchanged across the estrus cycle. The sleep-wake features of cycling females rodents and humans have been extensively studied (Yamaoka, 1980; Fang and Fishbein, 1996; Schwierin et al., 1998; Baker et al., 2001; Franken et al., 2006; Koehl et al., 2006; Paul et al., 2006; Andersen et al., 2008; Mong and Cusmano, 2016). While the findings have been somewhat inconsistent and some of the differences in findings on sleep-wake characteristics in those studies could be due to species and strain differences and differences in experimental designs, in general sleep-wake architecture does not seem to be substantially influenced by sex difference. Our findings that sleep-wake amounts and sleep-wake stability in cycling Fischer-344 females except for the proestrus phase are stable across estrus cycle are consistent with those studies.

Most notably, we found that the cycling females during proestrus night spent significantly less time in REM sleep compared to both estrus and ME-DE nights. This decrease in REM sleep amount was due to significantly decreased number of REM sleep episodes of all bout lengths. The REM sleep suppression during proestrus night was also significant compared to young males. We note that REM sleep suppression during the proestrus night is so remarkable that it could be used as a reliable marker of proestrus phase in young and probably middle aged female rats. While the significance of REM sleep suppression on the night of proestrus phase is not known, we speculate that it may be an adaptive necessity to avoid spending time in a condition with loss of muscle tone and spend more in active and alert state for mating with male. The following day, however, proestrus females spent significantly more time in REM sleep and also exhibited higher number of REM sleep episodes, especially during the early part of the day (see Figs. 2 and 3, G,H) as if animals were recovering from REM sleep loss of the proestrus night. The cycling females also spent more time in AW and less time in nonREM sleep during proestrus night.

Consistent with our findings, a suppression of sleep, especially REM sleep during proestrus night and seemingly its rebound the following day has also been reported in earlier studies in mice and rats (Yamaoka, 1980; Fang and Fishbein, 1996; Schwierin et al., 1998). In humans, subtle differences in sleep across menstrual cycle including REM sleep reduction during luteal phase is also well documented (Baker and Driver, 2007; Mong and Cusmano, 2016). Much evidence suggests that fluctuations in gonadal hormones in cycling females, at least in part, contribute to this REM sleep suppression during proestrus night. Gonadectomized male and female rodents do not exhibit significant differences in waking, nonREM sleep, and REM sleep amounts in either the dark or light phase (Paul et al., 2006; Mong and Cusmano, 2016). Progesterone and estradiol levels are highest during proestrus and ovariectomy, which diminishes the progesterone surge, attenuates sleep especially REM sleep suppression, whereas hormone replacement reinstates the suppression of both nonREM and REM sleep as seen in proestrus phase during the dark phase (Deurveilher et al., 2009, 2011; Schwartz and Mong, 2011; Deurveilher et al., 2013; Schwartz and Mong, 2013). In male rodents, however, castration does not alter sleep-waking compared to gonadally intact male rats and mice (Paul et al., 2006; Wibowo et al., 2012; Mong and Cusmano, 2016). Taken together, these studies suggest that sleep-waking is more sensitive to gonadal steroids in females and is resilient to fluctuations in testosterone levels in males (Mong and Cusmano, 2016).

Sleep is regulated by a network of sleep-active or sleep-facilitating neuronal groups that are distributed at several levels of the neuraxis and through their interactions with circadian and wake-promoting/facilitating neuronal networks (Saper et al., 2005; Moore, 2007; Alam, 2013; Alam et al., 2014; Thakkar et al., 2015; Chung et al., 2017; Jones, 2017; Van Erum et al., 2018). The underlying mechanism(s) mediating steroidal modulation of sleep-wake regulatory processes remains poorly understood. However, growing evidence suggests that ovarian steroids may directly act on sleep, wake, and/or circadian systems to modulate sleep-wake regulation in cycling females. For example, blocking oestradiol action in the median preoptic nucleus, a critical nonREM and REM sleep regulatory system, attenuates oestradiol-induced suppression of sleep (Mong and Cusmano, 2016). In ovariectomized females, oestradiol inhibits sleep-active ventrolateral preoptic area (VLPO) neurons and downregulates the mRNA and protein of the enzyme lipocalin-type prostaglandin D synthase (L-PGDS) responsible for the production of a somnogen, prostaglandin D2 (Mong et al., 2003; Hadjimarkou et al., 2008; Mong and Cusmano, 2016). Unlike females, fluctuations in testosterone in adult males do not influence activity of sleep-active neurons or L-PGDS protein levels in the VLPO, supporting a view that the male sleep–wake circuitry is less responsive to sex steroids (Mong et al., 2003; Hadjimarkou et al., 2008). Evidence suggest that sex differences in sleep are established by sex steroids during early development (Cusmano et al., 2014; Mong and Cusmano, 2016). Female neonatal rats exposed to masculinizing dose of testosterone exhibit male-like sensitivity to sex steroids in adulthood including the response of VLPO sleep-active neurons. Hypocretin neurons are wake-active and sleep-off, especially REM sleep off neurons. Evidence suggests that hypocretin neurons are not only sensitive to ovarian steroids rather higher levels of hypocretin could contribute to LH and prolactin surges as well as sleep suppression in proestrus (Porkka-Heiskanen et al., 2004; Silveyra et al., 2007; Mong and Cusmano, 2016).

In young and old Fischer-344 rats of both sexes, 6 h of sleep deprivation led to an increase in delta activity in nonREM recovery sleep, a function of prior-time waking and a measure of nonREM sleep intensity and its restorative function. While this increase in delta activity significantly decreased with aging, delta activity in nonREM recovery sleep in females was higher compared to males, across ages. An increased delta activity in nonREM recovery sleep and in some cases in baseline nonREM sleep in females has been reported in rats, mice, and humans (Armitage, 1995; Mourtazaev et al., 1995; Paul et al., 2006; Deurveilher et al., 2009, 2011; Schwartz and Mong, 2013; Mong and Cusmano, 2016). These studies indicate that the processes that regulate sleep propensity are sex-linked and are differently modulated in males and females, and in young females ovarian steroids potentially facilitate recovery from sleep loss. In one study, the effects of sleep deprivation were examined in the FCG mouse line, which comprised of four primary genotypes: XXF (XX mice lacking Sry, the testis determining gene, with ovaries), XYF (XY mice lacking Sry, with ovaries, XXM (XX mice with Sry and testes) and XYM (XY mice with Sry and testes). Following sleep deprivation, females with the XY compliment slept during their active phase and had higher nonREM sleep delta activity than XX females, suggesting that the processes mediating recovery from sleep loss are partially dependent on sex chromosomes (Ehlen et al., 2013). Evidence also suggests that ovarian steroids mediate facilitation of recovery sleep in a phase-dependent manner. Oestradiol administered to ovariectomized rats enhances REM sleep recovery as well as nonREM sleep delta activity during the light phase, while suppressing sleep in the dark phase (Schwartz and Mong, 2013). Currently, the effects of age and sex dependent alternations in hypothalamic–pituitary–gonadal axis on sleep-wake organization remain poorly understood.

Changes in sleep-wake organization with aging

Given that unlike old males, old females exhibit disturbance/cessation of the estrus cycle and reproductive senescence (Meites et al., 1976; Saksena et al., 1979; Goldman et al., 2007; Sone et al., 2007), one would expect that sex would be an important determinant of sleep-wake organization and the pathophysiology of sleep-wake regulations in aging. However, we found that the adverse effects of aging on sleep-wake amounts, their bout lengths and episode numbers, their circadian distribution, and homeostatic response to sleep loss were largely comparable in both old male and female rats. In brief, both old male and female rats exhibited: i) a

decrease in circadian amplitudes of sleep-wake states; ii) a decrease in AW amount and waking disruption by frequent sleep intrusions during the dark phase; iii) fragmented nonREM and REM sleep and their decreased amounts, and sleep disruption by frequent waking intrusions during the light phase; iv) decreases in delta activity in nonREM recovery sleep, although old females exhibited higher delta activity compared to old males. These findings are consistent with sleep-wake studies in human as well as other animal models including mice and other strains of rats, although, in one human study older men were found to have lighter sleep as indicated by reduction in N3 and REM sleep and higher arousal index (Dijk and Duffy, 1999; Mendelson and Bergmann, 1999; Redline et al., 2004; Blanco-Centurion and Shiromani, 2006; Wimmer et al., 2013; Mander et al., 2017; Kostin et al., 2019).

Evidence suggests that dysfunctional sleep- and wake-regulatory systems and a weaker signal for arousal from the SCN may contribute to sleep-wake instability with aging. Generally, aging-related abnormalities and/or neuronal loss has been linked to the dysfunction of critical sleep-, waking-, and circadian systems in the hypothalamus, locus coeruleus, and SCN (Mander et al., 2017). For example: i) hypocretin and noradrenergic neurons in the lateral hypothalamus and locus coeruleus, respectively in aged mice exhibit reduced fos protein immureactivity (Fos-IR), a marker of neuronal activation, in response to waking and increased endoplasmic reticulum stress and dyshomeostasis (Naidoo et al., 2013); ii) the number of hypocretin-immunoreactive neurons and wake-promoting effects of hypocretin decline with aging (Sawai et al., 2010; Kessler et al., 2011; Morairty et al., 2011); iii) hypocretin and noradrenergic neurons also exhibit lipofuscin accumulation (Panossian et al., 2011); iv) the amplitude of the day-night difference in SCN neuronal activity is reduced in old mice (Nakamura et al., 2011); v) sleep-active MnPO neurons exhibit declined response to homeostatic drive in aging (Alam et al., 2018); and vi) a paucity of galanin-immunoreactive intermediate nucleus neurons, the human homologue of the VLPO is accompanied by sleep fragmentation in older adults (Lim et al., 2014). Evidence suggests that increases in low-grade systemic inflammation, such as that accompanying aging, play a role in cellular aging including neuronal aging (Perry, 2010; Rosano et al., 2012; Zhang et al., 2013). Recently, we found that an attenuation or reduction of hypothalamic cell proliferation and neurogenesis, i.e., slowing of cell replenishment contributes to aging-like sleep-wake changes in young mice (Kostin et al., 2019). These findings, however, have not been fully investigated across sex lines.

It is pertinent to note some of the limitations of this study. Firstly, in this study as in most other studies, virgin animals were used and it remains to be determined if prior sexual experience and parity could affect steroidal modulation of sleep-wake control as observed for some brain functions. Also, the effects of previous sexual experience and pregnancy on age-related changes in sleep-waking remains poorly understood. Furthermore, like most earlier studies, in this study smear samples were collected just before dark-onset and based on the cytological analysis, a 24 h period was assigned to proestrus, estrus, and ME-DE phases, respectively, without due consideration to the ending of the cycles. Therefore, given the smaller sample size, a 24 h analysis may not truly reflect the associations between variabilities in ovarian steroidal levels and sleep-wake changes. More systematic studies addressing these issues are needed for a better understanding of the relationship between gonadal steroids and sleep-wake regulation and improving translational potential of the animal data to human subjects.

In summary, this study suggests that despite hormonal and other differences, the general characteristics of sleep-wake architecture in young females across estrus cycle are largely comparable except for REM sleep suppression during proestrus night and an increased REM sleep rebound during the following day. Compared to male, female rats also exhibit increased delta activity during nonREM recovery sleep. Aging significantly affects sleep-wake architecture as well as the homeostatic responses to sleep loss in both sexes. Changes with aging include, waking disruption and frequent sleep intrusions during the dark-phase; more fragmented and decreased amounts of nonREM and REM sleep, and sleep disruption by frequent waking intrusions during the light-phase; and decreased delta activity in nonREM recovery sleep in response to sleep deprivation. The mechanisms underlying sleep-wake disturbances in aging remains poorly understood but seems to be equally affected in both sexes.

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