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ORIGINAL ARTICLE

Sleep patterning changes in a prenatal stress model of depression

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Clinical depression is accompanied by changes in sleep patterning, which is controlled in a circadian fashion. It is thus desirable that animal models of depression mirror such diurnally-specific state alterations, along with other behavioral and physiological changes. We previously found several changes in behavior indicative of a depression-like phenotype in offspring of rats subjected to repeated, variable prenatal stress (PNS), including increased locomotor activity during specific periods of the circadian cycle. We, therefore, investigated whether PNS rats also exhibit alterations in sleep/wakefulness behavior around the change from light-to-dark phase. Control and PNS Sprague–Dawley rats were implanted with electrodes for continuous monitoring of electroencephalic activity used to determine behavioral state. The distribution of slow-wave sleep (SWS), rapid eye movement sleep (REMS) and wakefulness was compared for periods before and after lights were turned off, between baseline conditions and after exposure to an acute stressor. Both REMS and SWS amounts were increased in PNS rats relative to control animals in the beginning of the dark phase. REMS changes were due to an increase in REMS bout number, rather than in bout duration. During this circadian time period, we did not find any sex differences in the state changes. These results indicate that PNS affects baseline sleep patterning in both male and female rats around active-phase onset.

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Key words: acute stress, EEG, rat, REMS, slow-wave sleep

Introduction

A characteristic and diagnostic criterion for clinical depression is a change in sleep architecture.[1] Specifically, a reduced latency to onset of rapid eye movement sleep (REMS) and an increased duration of REMS are hallmark features of depression.[2] Moreover, abnormalities in slow-wave sleep (SWS) have also been reported.[3] Interestingly, a gender difference between sleep disturbances in major depressive disorder (MDD) has been noted.[4–6] For instance, in a study examining dizygotic twin pairs suffering from MDD, women experienced hypersomnia whereas men reported insomnia.[5]

Rats born to dams that have been exposed to stressors during the pregnancy display many behavioral and physiological traits that reflect emblematic changes in patients diagnosed with depressive disorders, including sleep changes.[7,8] For instance, prenatal stress (PNS) in rodents alters circadian rhythms, increases REMS, and heightens overall activity levels. In these animals, REMS could be normalized to control levels by administration of antidepressants.[7,9] Furthermore, PNS increases anxiety levels and changes coping strategies in rats,[10,11] indicating PNS as a valid depression model. While sex differences in the effect of PNS on behavior have been reported,[12,13] unfortunately, most rodent sleep studies have been conducted in males, limiting the knowledge about sex-specific sleep architecture differences as a consequence of PNS.[7,8,14] Therefore, we investigated whether PNS might impact sleep quality in a sex-dependent manner as well.

Acute stress in the absence of a depressive phenotype can also induce sleep changes, often in the form of REMS rebound.[15] However, acute stress can differentially influence behavior and neuroendocrine responses in PNS and control individuals in both humans and animals.[12,13,16] This indicates that PNS impacts mechanisms engaged to cope with stressful situations. Little is known about how exposure to both PNS and acute stress later in life influences sleep/wakefulness architecture.

We previously reported that increased ambulation and rearing behavior were induced predominantly around the onset of the dark phase in offspring of rats that had been subject to variable gestational stress.[13] Moreover, this circadian specificity in locomotor activity was increased in both male and female PNS rats.[13] We speculated that such activity changes may reflect a general reorganization of active *vs*. sedentary behavior, which potentially could result in alterations of sleep patterning at around the same circadian time point, that is, as the rats enter their active phase.

Therefore, the present PNS study examined the hypotheses that PNS impacts baseline sleep/wakefulness architecture around the vespertinal phase change, and that differential effects on state architecture are elicited by an acute stressor in

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PNS and control animals. We further examined the hypothesis that sleep/wakefulness architecture was affected differently in PNS females than in PNS male rats.

Our results showed that PNS rats had more REMS and SWS during the 3-h period after onset of the dark – that is, the early *active* phase. A REMS rebound effect was observed in control animals after exposure to an acute stressful event, which, however, was blunted in PNS animals. Nevertheless, our results did not indicate that sleep/wakefulness architecture was differentially altered in PNS female relative to PNS male rats in this time period.

Materials and methods

Animals and PNS paradigm

Virgin female Sprague–Dawley rats (weighing ~250 g) and male breeders were acquired (Charles-River, Sulzfeld, Germany) and used to generate control and PNS pups. Before maternal stress and behavioral testing of the offspring, the animals were housed under controlled conditions (12 h of light starting at 6 am, 20°C, 30–70% humidity) in cages with sawdust bedding and environmental enrichment. The rats had access to food and water *ad libitum*. After 5 days of acclimatization, each female was placed with a male rat until a positive vaginal smear indicated impregnation. Subsequently, the pregnant rats were housed individually. Half of the pregnant dams were exposed to a paradigm of repeated, variable stress throughout the period from gestational day (GD) 13–21, as previously described.[13] In brief, control and PNS dams were housed in separate rooms during the PNS paradigm period to prevent the behavior of PNS dams affecting control dams. The pattern of stressors consisted of two short-term stressors during the day (e.g. elevated platform and forced swimming) and one long-term stressor overnight (e.g. fasting or constant light). Exposure to the morning stressor started between 8.30 and 10 am and afternoon stressor exposure started between 1.30 and 3 pm. Exposure to the overnight stressor began immediately after completion of the last afternoon stressor. Control and stressor-exposed dams were weighed twice weekly to monitor weight gain during the pregnancy. The day the pups were born (usually on GD 22) was designated postnatal day (PND) 0. Pups were weighed on PND 2 and weekly thereafter until weaning on PND 22. After weaning, they were housed in groups of two or three. Male and female offspring were housed in separate housing units, but in the same room. Control and PNS offspring were surgically implanted with electrodes for electroencephalography (EEG) and electromyography (EMG) recordings around PND 55. All animal procedures were conducted in accordance with the Danish and EU legislation and approved by the national animal welfare committee (2012-15-2934-00153).

Surgical procedure and EEG sleep/wakefulness recordings

The rats were fitted with standard EEG and EMG electrodes under full anaesthesia. In brief, the rats were anaesthetized with a Hypnorm-Dormicum mixture (0.2 ml/100 g; H. Lundbeck A/S and Roche) and mounted in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA). A cortical electrode (E363-series; PlasticsOne, Roanoke, VA, USA) was attached above the right prefrontal cortex (coordinates in mm relative to bregma: anteroposterior (AP): +3.2; mediolateral (ML): −0.8) and a depth electrode (E363-series; PlasticsOne) was inserted into the CA1 region of the hippocampus (coordinates in mm: AP: −5.3; ML: −4.7; dorsoventral (DV): −2.6). A reference electrode (E363-series; PlasticsOne) was placed in the skull at AP: +8.0 mm and ML: −1.0 mm relative to bregma. An EMG electrode (E363-series; PlasticsOne) was inserted into the nuchal muscles and the wires from all electrodes were collected in a six-pin plastic connector (MS363; PlasticsOne) and fastened to the skull with dental acrylic cement (RelyX™, Unicem Aplicap™; 3M ESPE and Fuji PLUS; GC Europe). On the day of the surgery, and the subsequent 5 days, the animals were given antibiotic (Baytril®; Bayer) and anti-inflammatory (Rimadyl®; Pfizer) treatment. After the surgeries, the rats were housed individually to prevent injury to the rats as well as damage to the implants; 10–14 days after surgery (PND 65–74), the rats were placed individually in home-like cages in soundproofed EEG recording boxes with ventilation. A six-pin wire suspended from a rotating swivel was connected to the connector, allowing the animal to move freely within the recording box. The recording boxes, as well as the room within which they were placed followed the same 12-h light/dark cycle (lights on at 6 am) that the rats previously were accustomed to. Each box was equipped with food (Altromin #1324 and #1319; Brogaarden, Lynge, DK) and gel water (HydroGel, ClearH_2O; Portland, ME, USA). The analog EEG and EMG signals were amplified (Precision Model 440; Brownlee, Palo Alto, CA, USA) and converted to a digital signal (CED Power 1401, Power 1 (625 kHz, 16 bit) and CED Expansion ADC16; CED, Cambridge, England) at a sampling rate of 1 kHz. EEG and EMG signals were band-pass filtered at 0.75–100 and 30–300 Hz, respectively. A hardware notch filter (Precision Model 440, Brownlee) was applied at the power line frequency at 50 Hz for both EEG and EMG signals. The digitized signals were recorded using Spike2 (CED, Cambridge, England) and imported into NeuroScore (DSI, New Brighton, MN, USA) for analysis. The EMG signal was further processed by a high pass filter of 50 Hz, resulting in a bandwith of 50–300 Hz. The animals were housed in the EEG recording boxes for 5 days with continuous recording of EEG and EMG signals. Aside from being monitored via video recordings that were checked (between 3 and 4 pm), the animals were also physically inspected every morning (between 8 and 9 am) to ensure their well-being and that sufficient amount of food and gel water was available. Animals were weighed before and after the 5-day recording period.

Sleep/wakefulness behavior was analyzed for selected time periods. Baseline sleep was assessed for the time period 3–9 pm on day 3, that is, after 2 days of habituation to the EEG recording box. On day 4, the rats were exposed to an acute stressor by being placed on an elevated platform for 30 min. This stressor procedure was conducted immediately following the morning routine inspection, and entailed disconnecting the rat from the electrode leads, removing it from the recording box, and placing it on the elevated platform. Subsequently, the

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animals were returned to their respective recording boxes, reconnected and recordings continued. Sleep/wakefulness behavior was analyzed for the 3-h period after exposure to the acute stressor to evaluate whether this challenge influenced immediate sleep/wakefulness patterning in PNS animals differently than in the control group. The 3-h time period after exposure to the acute stressor was chosen because behavioral and neuroendocrine changes have been reported for this interval.[12,17,18] In addition, sleep/wakefulness behavior was also analyzed between 3 and 9 pm on day 4 (~6–12 h after stressor exposure, and corresponding to the baseline period) to investigate whether exposure to the acute stressor would alter sleep patterning during this interval.

Post hoc *signal conditioning and sleep scoring*

For sleep/wakefulness scoring, the recordings were divided into epochs of 10 s and states of sleep/wakefulness were manually assigned in accordance with other rodent sleep studies.[9] Each epoch was classified as representing either wakefulness, SWS or REMS using standard criteria. See Fig. 1 for examples of typical EEG and EMG signals, characteristic of each vigilance state. In brief, wakefulness was identified when >50% of the epoch contained a medium (quiet wake) to high (active wake) muscle tonus (EMG amplitude), and/or a mixed frequency of relatively low-amplitude (desynchronized) cortical EEG. SWS was scored when >50% of the epoch contained synchronized EEG waveforms, that is, a relatively high amplitude and low frequency, in combination with low and steady EMG signal. REMS was scored when >50% of the epoch contained low-amplitude EEG with higher and mixed frequencies (primarily θ waves; 4–8 Hz) and low-level EMG tonus (typically lower than seen during SWS). As an auxiliary measure, a derived signal of θ power (4–8 Hz) divided by δ power (0.75–4 Hz) was calculated for each epoch from the hippocampal depth electrode EEG signal. Increased (theta power/delta power) ratio was used as a confirmatory indicator for classifying an epoch as REMS. All rats were manually scored by C.S. who was blind to the animal's status (i.e. prenatal treatment; control *v.* PNS). Time spent in wakefulness, SWS and REMS was quantified for each analysis period. Furthermore, latency to onset of the first sleep episode (defined as ⩾30 s of SWS duration) was determined. Two separate variables of latency to REMS (⩾20 s REMS) were quantified: (i) latency to onset of REMS from exposure to the acute stressor and (ii) latency to REMS onset after first onset of sleep, thus subtracting initial wakeful periods. Number and average duration of uninterrupted bouts of REMS, SWS and wakefulness were quantified. Finally, arousals during SWS were quantified, and characterized as a period of wakefulness of ⩽30 s within a bout of SWS. All data measures were extracted blinded (both with respect to sex and treatment) with a custom made data processing and parsing pipeline.

Fig. 1. Representative EEG and EMG signals from one rat to illustrate the different sleep/wakefulness stages; high EMG combined with desynchronized, low-amplitude EEG identifies wakefulness (W), low EMG and synchronized high-amplitude EEG indicates slow-wave sleep (SWS), and finally, low EMG combined with desynchronized low-amplitude EEG corresponds to rapid eye movement sleep (REMS).

Statistics

All statistical analyses were performed with *R* (R Core Team, Vienna, Austria).[19] In general, if there was no effect of sex, data for males and females within each treatment group were pooled. The specific type of statistical test employed for each analysis is indicated in the Results section. The following procedure was used for each of the variables of interest. Normality was assessed using a Shapiro–Wilk test ($P > 0.05$). If normality could not be assumed, the variable was transformed by one of the following formulas: log(*y*), sqrt(*y*) or asin(sqrt(y/100)) depending on the apparent distribution (if a data transformation was performed, it is indicated in the Results section in parenthesis) – and re-checked for normality using the Shapiro–Wilk test. If homogeneity of the variances, assessed by Bartlett's test ($P > 0.05$), could be assumed, data were analyzed by a two-way ANOVA with treatment (PNS *vs.* control) and sex (male *vs.* female) as factors.

To evaluate the impact of the acute stressor (i.e. when sleep/wakefulness behavior was compared across days), a three-way ANOVA was performed, with sex and treatment as between-subject variables and day as a within-subject variable. To directly visualize the effect of the acute stressor, data were normalized relative to their respective time-locked baseline (day 3) measures in the control group (i.e. the control group at baseline is set to 100%). Therefore, the relative distribution of wakefulness, SWS and REMS on day 4 (time period 3–6 pm) for each group was compared with baseline measures in the corresponding group of day 3 (time period 3–6 pm). Likewise, the relative distribution of wakefulness, SWS and REMS on day 4 (time period 6–9 pm) for each group was compared with baseline measures in the corresponding group of day 3 (time period 6–9 pm). Moreover, the PNS group at baseline (day 3, time period 3–6 pm) was compared with the control group on day 3 (time period 3–6 pm). The PNS group at baseline (day 3, time period 6–9 pm) was compared with the identical time period of the control group (day 3, time period 6–9 pm).

Data sets for which normal distribution could not be assumed (even after transformations), were analyzed using a Kruskal–Wallis rank sum test (KWRST). This test was performed using one main effect at a time (sex followed by treatment).

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For all analyses, a *P*-value of 0.05 was used as the significance level. All values are presented as means ± standard error of mean (S.E.M.).

Results

We used 27 female (15 control and 12 PNS) and 23 male (11 control and 12 PNS) rats derived from nine control and six PNS dams for this sleep/wakefulness study. To minimize litter effects, a maximum of three offspring of each sex was included from any litter. PNS did not affect gross development of the rats as we observed no significant differences in the weight of neither male nor female pups between PNS and control offspring (data not shown), which is in agreement with our previous findings.[13]

Consequences of PNS on baseline sleep state distribution

Sleep and circadian rhythm disturbances are hallmarks of depression, and we aimed to investigate how PNS impacted baseline sleep/wakefulness distribution around the switch from light-to-dark phase (i.e. 3–9 pm) in male and female rats. During the first 3 h of the dark phase, PNS rats ($n = 21$), when compared with control rats ($n = 24$) irrespective of sex, spent significantly more time in REMS and SWS [REMS: sqrt(y), ANOVA, $F(1,41) = 9.539$, $P = 0.004$; SWS: log(y), ANOVA, $F(1,41) = 5.757$, $P = 0.021$, respectively] with a corresponding drop in time spent awake [Fig. 2, right panel; wakefulness: asin(sqrt(y/100)), ANOVA, $F(1,41) = 9.800$, $P = 0.003$]. The amount of time spent in REMS was 6.4 ± 0.9% in control and 11.7 ± 1.4% in PNS animals during this period. No sex differences in relative time spent in REMS [sqrt(y), ANOVA, $F(1,41) = 0.511$, $P = 0.479$], SWS [log(y), ANOVA, $F(1,41) = 0.021$, $P = 0.885$] or wakefulness [asin(sqrt(y/100)), ANOVA, $F(1,41) = 0.069$, $P = 0.794$] were observed for this time period. In contrast, males irrespective of prenatal treatment ($n = 24$) spent more time in REMS than females ($n = 21$) during the last 3 h of the light phase [12.9 ± 0.6 and 9.9 ± 0.7%, respectively; ANOVA, $F(1,41) = 9.094$, $P = 0.004$]. There was, however, no significant effect of PNS on sleep/wakefulness distribution [REMS: ANOVA, $F(1,41) = 0.114$, $P = 0.737$; SWS: KWRST, χ^2(df:1) = 0.634, $P = 0.426$; wakefulness: log(y), ANOVA, $F(1,41) = 0.113$; $P = 0.739$] during this time period (Fig. 2, left panel).

Number and average duration of sleep bout at baseline

We examined whether the increase in REMS during the early dark phase was due to increased duration or number of individual REMS periods. PNS ($n = 21$) was associated with a significant increase in the number of REMS bouts [ANOVA, sqrt(y), $F = (1,41) = 6.505$, $P = 0.015$, Fig. 3a] when compared to that in control rats [$n = 24$; data pooled across sexes due to lack of sex differences; ANOVA, sqrt(y), $F(1,41) = 0.057$, $P = 0.813$]. In contrast, we saw no significant effects of treatment [KWRST, χ^2(df:1) = 0.787, $P = 0.375$] or sex [KWRST, χ^2(df:1) = 0.081, $P = 0.776$] on the average REMS bout duration during the first 3 h of the dark phase (i.e. 3–9 pm, Fig. 3d). We found no significant differences in REMS bout numbers as a consequence of treatment [ANOVA, $F(1,41) = 1.232$, $P = 0.273$] or sex [ANOVA, $F(1,41) = 0.166$, $P = 0.686$] for the last 3 h of the light phase. However, females ($n = 24$; irrespective of treatment) exhibited shorter REMS bout duration relative to males ($n = 21$) at 3–6 pm [ANOVA, $F(1,41) = 14.5586$, $P = 4.5 \times 10^{-4}$; Fig. 3d].

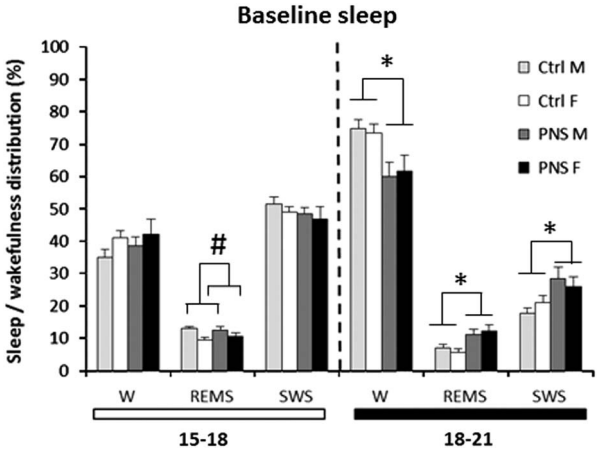

Fig. 2. Effects of prenatal stress on sleep/wakefulness distribution around the light-to-dark phase transition (18–21 pm). Baseline sleep/wakefulness distribution was assessed in control (Ctrl) and prenatally stressed (PNS), male (M) and female (F) rats after ~54 h of habituation to the recording box. The relative time spent in rapid eye movement sleep (REMS), slow-wave sleep (SWS) and wakefulness (W) was evaluated in home-like cages. We evaluated sleep/wakefulness distribution for two time periods: (i) the 3-h period just before lights were turned off, that is 15–18 pm and (ii) the first 3 h after lights were off, that is 18–21 pm (indicated by light and dark horizontal bars, respectively, below histograms). Results are presented as mean ± S.E.M., $n = 10$–14. A number sign (#) denotes a significant sex difference and an asterisk (*) marks a significant difference between PNS and control rats for the corresponding time periods, $P < 0.05$.

Even though PNS significantly increased the total amount of time spent in SWS in the beginning of the dark phase, this did not manifest as a significant change in either number [PNS: 44 ± 3; control: 39 ± 4; ANOVA, $F(1,41) = 0.848$, $P = 0.362$] or duration of SWS bouts [PNS: 70 ± 5; control: 63 ± 5; ANOVA, $F(1,41) = 1.146$, $P = 0.291$; Fig. 3b and 3e]. During the last part of the light phase (i.e. 3–6 pm), PNS animals ($n = 21$), irrespective of sex, exhibited a reduced number of wakefulness bouts relative to control rats [$n = 24$; 64 ± 4 *v.* 74 ± 2, ANOVA, $F(1,41) = 5.601$, $P = 0.023$, Fig. 3c]. However, we did not find a significant effect of treatment [KWRST, χ^2(df:1) = 1.143, $P = 0.285$] or sex [KWRST, χ^2(df:1) = 3.652, $P = 0.056$] on average bout duration of wakefulness during the 3–6 pm time period, Fig. 3f. In the beginning of the dark phase (i.e. 6–9 pm), the reduction in wakefulness percentage in PNS animals (Fig. 2) was accompanied by a shorter wakefulness duration [KWRST, χ^2(df:1) = 4.100, $P = 0.043$], rather than a significant change in the number of bouts spent in wakefulness [ANOVA, treatment: $F(1,41) = 0.713$, $P = 0.4033$; Fig. 3c and 3f].

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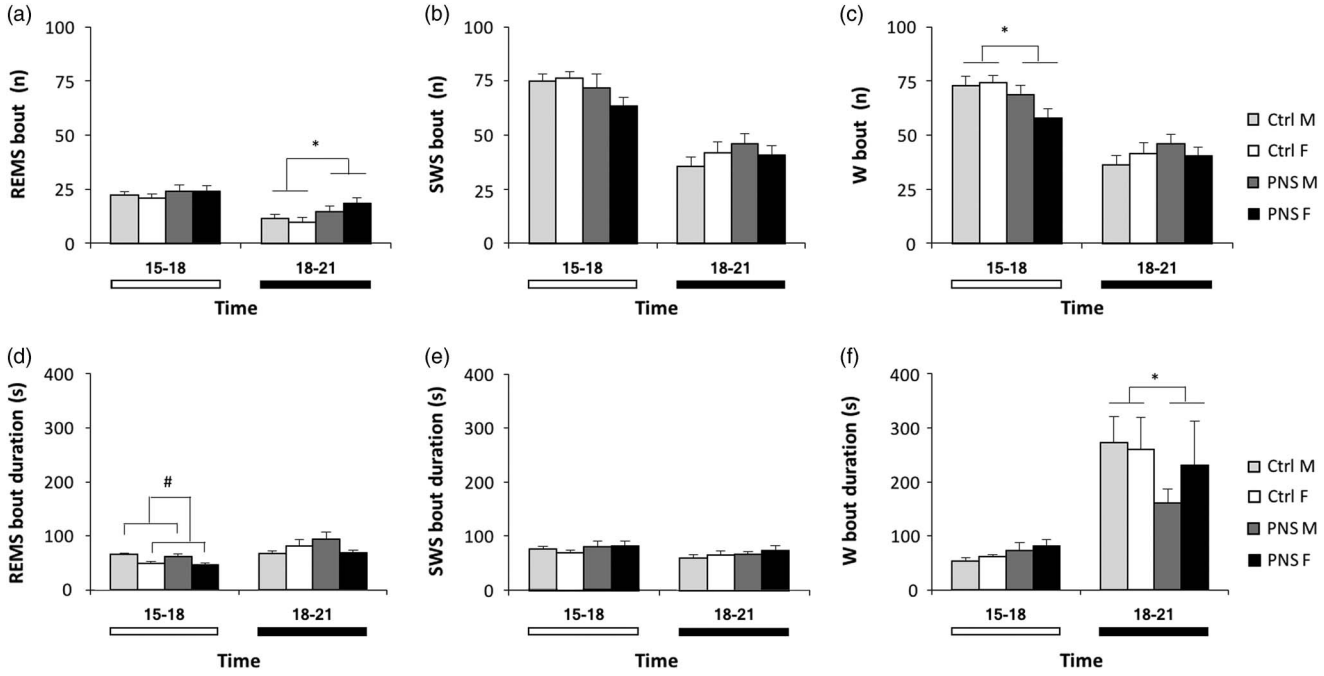

Fig. 3. Prenatal stress effects on rapid eye movement sleep (REMS), slow-wave sleep (SWS) and wakefulness (W) bout number and duration around the lights-off phase-shift. Number of REMS (*a*), SWS (*b*) and wakefulness (*c*) bouts as well as duration of REMS (*d*), SWS (*e*) and wakefulness (*f*) bouts were quantified in control (Ctrl) and prenatal stress (PNS) rats for two time periods: the 3-h period before and after, lights-off, respectively (indicated by light and dark horizontal bars below each histogram). Results are presented as mean ± S.E.M., $n = 10$–14, number sign (#) denotes a significant sex difference and an asterisk (*) marks a significant difference between PNS and control rats for the corresponding time periods, $P < 0.05$.

Sleep structure during the first 3 h after exposure to an acute stressor

To assess whether acute stressor exposure alters sleep differentially in PNS rats, we placed both PNS and control rats on an elevated platform for 30 min and subsequently evaluated the immediate changes in sleep structure. PNS animals ($n = 22$), irrespective of sex, exhibited increased duration of wakefulness bouts relative to control rats [$n = 21$; KWRST, χ^2(df:1) = 4.067, $P = 0.044$], whereas the number of wakefulness bouts was not significantly different between treatment groups immediately after acute stress [ANOVA, $F(1,39) = 3.481$, $P = 0.070$; Fig. 4a]. Despite these wakefulness bout alterations, PNS animals did not spend significantly more total time awake than control rats during the first 3 h after stressor exposure [ANOVA, $F(1,39) = 3.398$, $P = 0.073$; Fig. 4a]. We did not observe sex differences in the distribution of wakefulness for the first 3-h period after exposure to the acute stressor [ANOVA, $F(1,39) = 0.000$, $P = 0.993$; data not shown].

Exposure to the acute stressor resulted in a significantly lower number of SWS bouts (~20%) in PNS animals ($n = 22$) relative to control rats ($n = 21$) in the first 3-h period immediately after exposure to the acute stressor [ANOVA, $F(1,39) = 5.005$, $P = 0.031$]. We saw no sex differences in this response [ANOVA, $F(1,39) = 0.003$, $P = 0.956$; data not shown]. Moreover, PNS animals ($n = 22$) had fewer arousals during SWS than control rats [$n = 21$; ANOVA, $F(1,39) = 4.367$, $P = 0.043$, Fig. 4b]. However, we saw no significant effect of PNS on SWS bout duration [log(y), ANOVA, $F(1,39) = 0.468$, $P = 0.498$], nor on percent time spent in SWS just after exposure to acute stressor [ANOVA, $F(1,39) = 3.664$, $P = 0.063$; Fig. 4b]. Exposure to an acute stressor did not significantly impact sleep latency [log(y), ANOVA, $F(1,39) = 2.214$, $P = 0.145$], which on average was 20.0 ± 2.2 min for control animals ($n = 21$) and 29.4 ± 4.5 min for PNS animals ($n = 22$; data not shown). Similarly, we found no significant differences in REMS latency after acute stress between PNS and control animals. This was the case both when REMS latency was measured from exposure to the acute stressor [log(y), ANOVA, treatment: $F(1,39) = 0.359$, $P = 0.553$; sex: $F(1,39) = 0.349$, $P = 0.558$], and when measured from first sleep onset [log(y), ANOVA, treatment: $F(1,39) = 0.099$, $P = 0.755$; sex: $F(1,39) = 0.758$, $P = 0.389$], regardless of treatment and sex (Fig. 4c).

We did observe sex differences in the amount of time spent in REMS as well as the REMS bout duration following the acute stressor. Specifically, male rats ($n = 22$), irrespective of prenatal treatment, spent significantly more time in REMS during the first 3 h after exposure to the acute stressor than female rats [$n = 21$; 8.4 ± 0.7 *vs.* 6.2 ± 0.7%, ANOVA, $F(1,39) = 4.885$, $P = 0.033$]. Furthermore, male rats ($n = 22$), irrespective of

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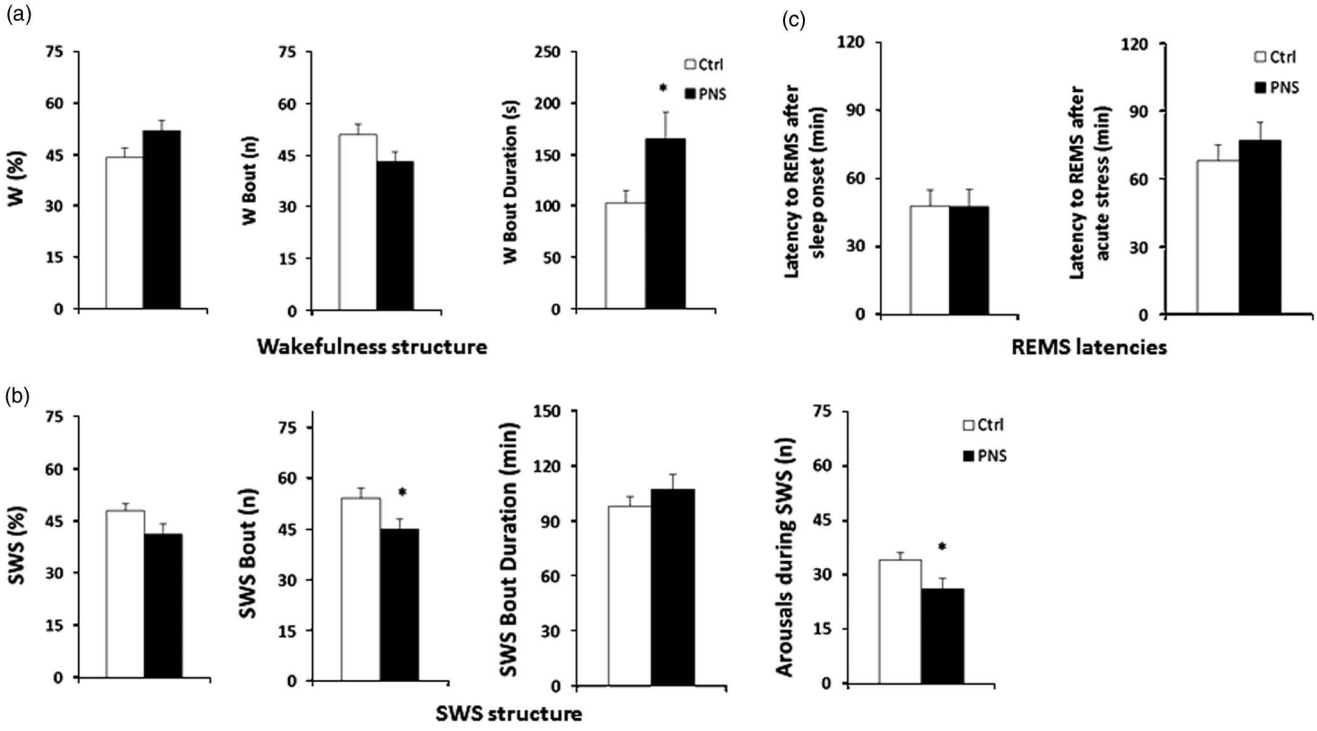

Fig. 4. Sleep/wakefulness is minimally impacted just after acute stress. Sleep/wakefulness variables in control (Ctrl, white bars) and prenatal stress (PNS, black bars) rats were assessed for the first 3 h following acute stress (30 min placement on an elevated platform). Time spent in, bout numbers of, and mean bout duration of (*a*) wakefulness (W) and (*b*) slow-wave sleep (SWS), as well as prevalence of arousals during SWS were quantified. The latter measure was defined as periods of wakefulness lasting ⩽30 s inbetween SWS bouts. (*c*) Latency to onset of rapid eye movement sleep (REMS) from sleep onset and REMS latency after exposure to acute stressor were also quantified. Results presented are mean ± S.E.M., $n = 21$–22, and an asterisk (*) marks a significant difference from control rats ($P < 0.05$). Data were pooled across sexes as there were no significant sex differences in PNS effects.

prenatal treatment, had a longer REMS bout duration than did female rats [$n = 21$; 73 ± 5 *vs.* to 60 ± 5 s, ANOVA, log(y), $F(1,39) = 4.870$, $P = 0.033$], whereas the number of REMS bouts was similar in males and females (13 ± 1 *v.* 12 ± 2).

Sleep/wakefulness structure ~6–12 h after exposure to the acute stressor

We further investigated sleep/wakefulness structure around the onset of the rats' active phase (i.e. 3–9 pm), which was the same time period analyzed for baseline sleep patterns (on day 3). Similar to findings obtained when analyzing the same time period the day before, the distribution of REMS, SWS and wakefulness during the last 3 h of the light phase (i.e. 3–6 pm) was not affected by PNS treatment [REMS: ANOVA, $F(1,39) = 0.246$, $P = 0.623$, Fig. 5a; SWS: ANOVA, $F(1,39) = 1.113$, $P = 0.298$, Fig. 5b; wakefulness: ANOVA, $F(1,39) = 1.121$, $P = 0.296$, Fig. 5c].

In the beginning of the dark phase, PNS animals exhibited an increased percentage of REMS relative to control rats [ANOVA, $F(1,39) = 13.722$, $P < 6.6 \times 10^{-4}$, Fig. 6a] which was similar to the findings on the baseline day. Similarly, the increase in SWS observed in PNS animals when compared to that seen in control rats, irrespective of sex, in the dark phase at baseline (i.e. on day 3) remained after acute stress on day 4 [136% SWS at baseline and 145% after acute stress; ANOVA, treatment: $F(1,39) = 8.810$, $P = 0.005$, Fig. 6b]. This was also the case for reductions in wakefulness in the beginning of the dark phase when compared with reductions exhibited by control rats during the same period [ANOVA, $F(1,39) = 14.603$, $P = 4.654 \times 10^{-4}$, Fig. 6c]. When comparing the variables after acute stress to the values obtained at the same time period on the baseline day (6–9 pm; Fig. 6), REMS percentage was increased in control and PNS animals to 167.9 ± 11.0 and 240.5 ± 15.6%, respectively. The increase as an effect of the acute stressor was larger in controls (168/100 = 1.68-fold increase), compared with PNS animals (240/182 = 1.32-fold increase) when considering that in the PNS group on the baseline day, REMS percentage was already increased to 182.3 ± 21.5% relative to control levels.

To directly investigate whether effects of acute stress were statistically significant, the amount of time spent in REMS during the dark phase was compared between days, using a three-way ANOVA [treatment: $F(1,78) = 22.088$, $P = 1.1 \times 10^{-5}$; sex; $F(1,78) = 0.0146$, $P = 0.703$; day: $F(1,78) = 0.865$, $P = 0.355$; treatment × day: $F(1,78) = 0.023$, $P = 0.879$; sex × treatment: $F(1,78) = 0.270$, $P = 0.605$; sex × treatment × day: $F(1,78) = 0.160$, $P = 0.690$]. Only a significant treatment main effect was obtained, whereas surprisingly, the treatment × day

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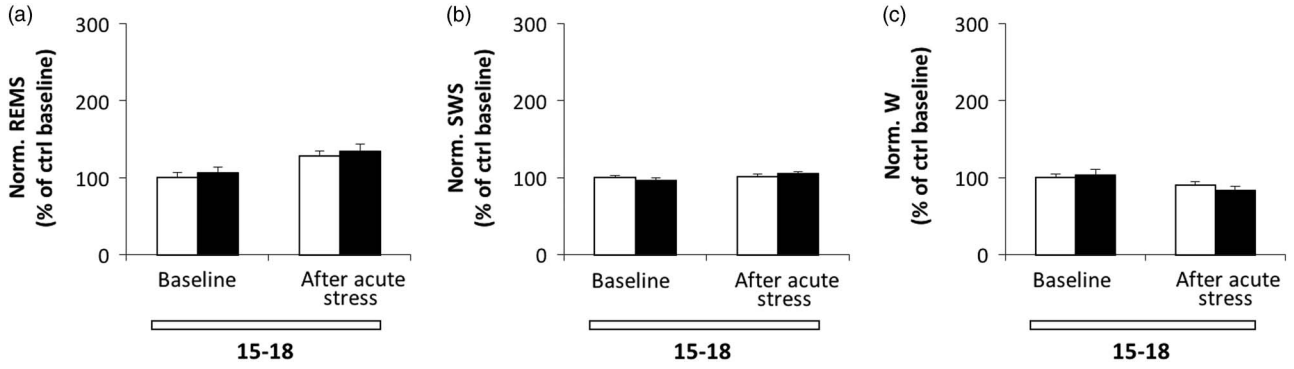

Fig. 5. No impact of acute stress on sleep/wakefulness parameters during 15–18 pm were detected. Sleep/wakefulness variables were compared around the lights-off phase-shift between control (Ctrl, white bars) and prenatal stress (PNS, black bars) rats at baseline (day 3) and after exposure to an acute stressor (day 4). Time spent in (*a*) rapid eye movement sleep (REMS), (*b*) slow-wave sleep (SWS) and (*c*) wakefulness (W) was normalized to baseline levels in control rats at 15–18 pm for each vigilance state (phase indicated by a light bar below each histogram). Results presented are mean ± S.E.M., $n = 21–22$.

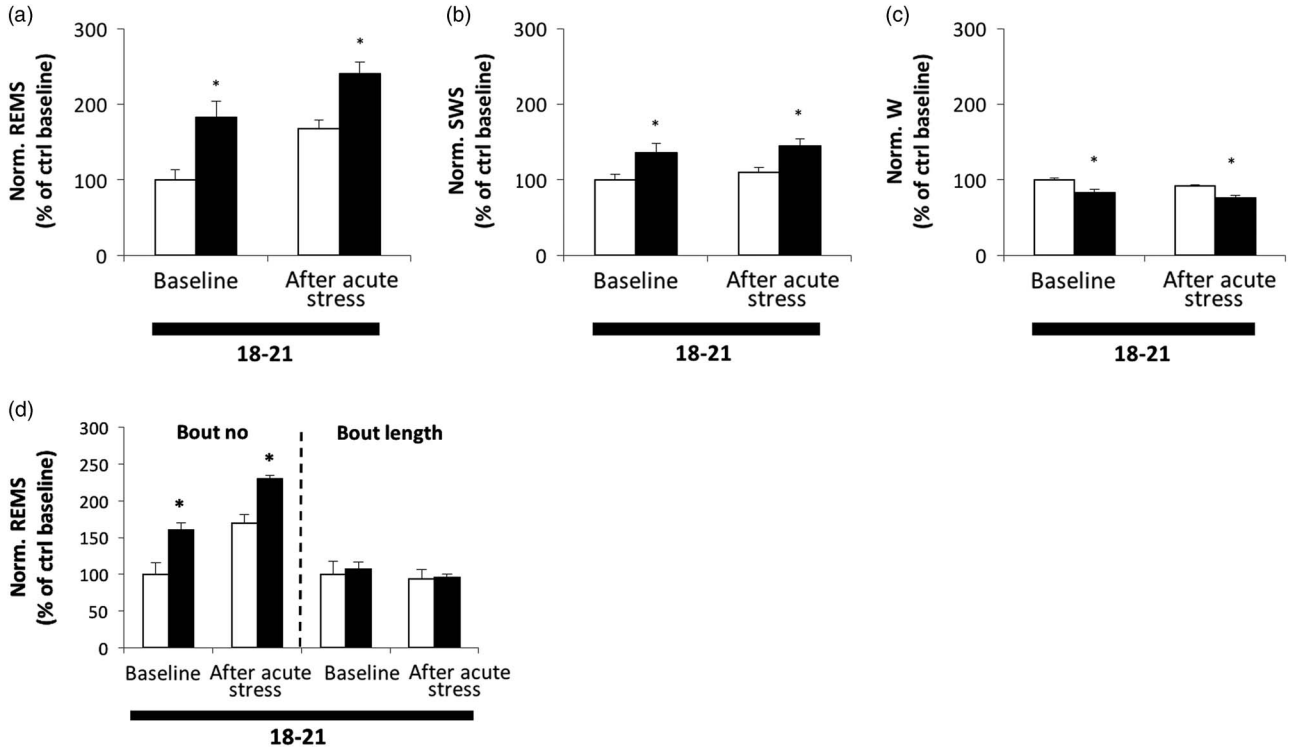

Fig. 6. Acute stress impacts sleep/wakefulness parameters during 18–21 pm. Sleep/wakefulness variables were compared around the lights-off phase-shift between control (Ctrl, white bars) and prenatal stress (PNS, black bars) rats at baseline (day 3) and after exposure to an acute stressor (day 4). Time spent in (*a*) rapid eye movement sleep (REMS), (*b*) slow-wave sleep (SWS) and (*c*) wakefulness (W), as well as (*d*) bout numbers (left panel) and bout duration (right panel) were normalized to baseline levels in control rats at 18–21 pm for each vigilance state (phase indicated by a dark bar below each histogram). Results presented are mean ± S.E.M., $n = 21–22$. An asterisk (*) indicates significant difference from control rats on the same day of recording ($P < 0.05$). Data were pooled across sexes as there were no significant sex differences in PNS effects.

interaction did not reach significance – thus precluding further specific comparison of treatment groups between days.

In the dark phase (6–9 pm), the number of REMS bouts after acute stress was increased to 161.1 ± 11.0% for controls and 221.7 ± 12.9% for PNS animals, compared with control levels in the same time period on the baseline day. PNS animals had 57.9 ± 18.0% more number of REMS bouts on the baseline day when compared with controls. Exposure to acute stress resulted in a ~ 1.61-fold increase in REMS bout numbers in the control animals. In PNS, animals on the other hand, REMS bout

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numbers were only increased by a factor of ~ 1.40 as an effect of acute stress, potentially because of the higher number of REMS bouts at baseline. However, three-way ANOVA again only revealed a significant treatment main effect [$F(1,78) = 15.017$, $P = 2.21 \times 10^{-4}$], whereas the remaining main and interaction effects did not reach significance [sex: $F(1,78) = 0.029$; $P = 0.865$; day: $F(1,78) = 0.378$, $P = 0.541$; sex × treatment: $F(1,78) = 0.000$; $P = 0.988$; sex × day: $F(1,78) = 0.965$, $P = 0.329$; treatment × day: $F(1,78) = 0.093$, $P = 0.761$; sex × treatment × day: $F(1,78) = 1.802$, $P = 0.183$] and thus did not permit further *post hoc* analysis.

In summary, minor changes in sleep/wakefulness architecture were observed in the 3-h period immediately after acute stress. Furthermore, following acute stress, PNS animals demonstrated increased SWS and reduced wakefulness in the beginning of the dark phase – an effect similar in magnitude to these variables differences on the baseline day. While there was a further increase in REMS% and REMS bout number after acute stress, which were less pronounced in PNS animals relative to the increases seen in control animal, these latter effects of exposure to acute stress did not reach statistical significance.

Discussion

Depressive disorders are often associated with changes in the balance of sleep and wakefulness states.[3,20,21] In the present study, we examined whether changes in sleep patterning were present in the PNS model of depression around active-phase onset. We focused the analysis on this circadian phase change since we previously found other behavioral changes in PNS rats during this period.[13] Furthermore, given that females are more susceptible to depression,[21,22] we tested whether sex differences in sleep/wakefulness balance existed in the PNS animals. Our results demonstrate that repeated variable PNS does alter sleep architecture in young adult offspring. Sleep/wakefulness changes were apparent at the beginning of the dark phase, when PNS rats exhibited an increase in the relative amount of time spent in SWS, as well as REMS. Furthermore, we found that the increase in REMS was due to an increased number of REMS bouts, rather than changes in the average duration of REMS bouts. We saw baseline changes in sleep/wakefulness patterning between male and female rats REMS and measures increased selectively in male rats only after acute stressor exposure, however, this occurred in both control and PNS groups.

Sleep/wakefulness architecture at baseline

Circadian rhythms are altered in depressed patients.[23] For example, MDD patients exhibit a phase shift in their sleeping behavior.[24] Consistent with this finding, PNS rats show alterations in behaviors controlled in a circadian fashion. PNS rats show heightened increases in locomotor and rearing activity particularly around the change from the light phase to the dark phase.[13] As we had previously seen a light-to-dark transition behavioral alteration in PNS rats, we investigated sleep/wakefulness architecture during this time period. Changes in sleep/wakefulness variables as a consequence of PNS were less evident in the last part of the light phase relative to the subsequent beginning of the dark phase. In the beginning of the dark phase, which is the circadian period with the highest degree of activity in the rat, PNS animals had increased REMS and SWS sleep. Increases in REMS have also been documented in other PNS animal studies,[7,9,14] as well as for the effects of chronic mild stress in adult rats[25] – another widely used animal model of depression.[26] In general, however, experimental differences in time periods utilized in previous studies from those used in our study precludes a detailed comparison of changes in REMS prevalence. Nevertheless, our data show that REMS is altered in PNS rats and indicate that REMS changes present an association with the circadian phase.

In addition to finding an increase in REMS at the beginning of the dark phase, we also found an increase in SWS (corresponding to human NREM sleep) in PNS animals. In depressive patients, a general decrease in NREM has been reported.[27,28] Another PNS rat study found a concomitant increase in SWS1 and REMS (and a lower ratio of SWS2) at the expense of wakefulness.[7] Whereas the increase in REMS was persistent throughout the day in the aforementioned study, the changes in wakefulness and SWS were restricted to the dark phase. However, these authors did not report whether total SWS was significantly higher in the PNS than control group. Mairesse *et al.*[9] also did not distinguish between light and deep SWS, but reported significantly less overall SWS in animals exposed to prenatal restraint stress. These inconsistencies indicate that, contrary to the rather persistent finding of increase in REMS, the changes in SWS might be very dependent on the specific circadian period studied.

Taken together, our past and present findings suggest that PNS animals are both more active[13] and spend more time in REMS and SWS around dark phase onset. This suggests that while the PNS animals sleep more at this circadian phase period, when they are awake, they are more active than control rats. Alternatively, our findings may be explained by the different experimental conditions utilized across our studies. In the locomotor assessments of PNS animals, subjects were tested in the vicinity of other rats, whereas in the sleep studies, the rats were isolated to ensure that the animals did not influence each other. Nevertheless, while we cannot discern whether PNS animals are more active than control rats and/or whether social factors play a role in heightened activity, it is clear that under both experimental situations, PNS and control animals responded differently at this circadian period.

PNS did not appear to affect the latency to onset of REMS after the lights were turned off. A decreased REMS latency is one of the most commonly reported sleep changes in depressed patients[3,28–30] and is also reported for animal models of depression.[14,25,31] However, some studies of depressed patients did not detect a difference in REMS latency,[20,32] indicating that this effect may be context specific or develop only in a subpopulation of depressed patients. Many sleep studies do not

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include quantification of REMS latency and those that do, report contrasting results. One limitation is that the adopted definition of REMS latency in the literature is rather diverse,[2,14,30,33] which complicates comparison across studies. In the present study, we therefore examined two types of REMS latency; REMS latency after a zeitgeber (lights off or exposure to acute stressor) and REMS latency after first onset of sleep after the zeitgeber (i.e. eliminating initial bouts of wakefulness). Either way, we found no difference in REMS latencies as a consequence of PNS.

Impact of acute stress on sleep/wakefulness architecture

Often, a stressful event later in life, in addition to early adverse effects (e.g. PNS) or other predisposing factors, can act synergistically to trigger a depression-like phenotype. In the case of exposing rats to PNS, we previously found that locomotor activity measured in an elevated plus maze was significantly altered in control animals after exposure to an acute stressor – however, this effect was blunted in PNS animals.[13] The impact of exposure to an acute stressful episode on sleep architecture, as well as the persistence of these alterations, varies between studies. For instance, acute restraint stress increases REMS time immediately after stressor exposure[34,35] as well as 1 day, but not 10 days, after stressor exposure.[34] In contrast, 2 h of social stress during the dark phase reduced time spent in REMS in the first 2 h. However, it was not heightened in the subsequent 4 h after stress,[36] which indicates that stress affects subsequent sleep differentially, depending on the time period investigated. A commonly found effect after exposure to stressful episodes is REMS rebound.[35] Dugovic *et al.* found a general increase in time spent in REMS after 1 h of restraint stress in both control and in PNS rats. In the present study, PNS rats exhibited 50% longer wakefulness bout duration relative to control animals during the first 3 h after acute stress.[7] However, our PNS animals did not exhibit longer sleep latencies after the elevated platform stressor, nor were REMS latencies altered.

Nevertheless, total REMS time in control animals was increased by ~68% after acute stress (relative to baseline the day before) – specifically during the beginning of the dark phase. It has been suggested that REMS rebound is a protective and adaptive mechanism to cope with stressful/aversive stimuli,[15] but may also be a response to mild sleep deprivation,[27] and our acute stressor was presented during the light/rest phase. We did not observe a difference in the increase of overall REMS after acute stress between PNS and control rats. This finding appears to contrast with that of Dugovic *et al.*[7] However, the latter study was conducted over a different time period in somewhat older animals, which may partly explain these inconsistencies. Furthermore, differences in the PNS stressor paradigm may also contribute to the different outcome. However, discrepancies in the timing of state observations relative to stressor presentation and circadian phase is a very likely source of divergence of results, given how dependent the state alterations are on these factors.

Impact of sex on sleep/wakefulness patterns

In contrast to what has previously been reported in patients with MDD,[23,24] we observed no sex differences in the examined sleep/wakefulness variables between control and PNS rats under baseline conditions, nor after acute stress. These data suggest that the sex-specificity of behavioral changes observed in rodent depression models does not include alterations of sleep architecture. However, we did not monitor, nor correct for, the hormonal cycle in the females and it remains to be established if this, at least partly, obscures sex differences in state-patterning.

Implications of findings

We have identified several sleep variables that are significantly different in PNS rats compared to control rats, around onset of the rats active phase. Although sleep variables differed between male and female rats both under baseline conditions and immediately after acute stress, this was the case for control as well as PNS animals. However, our findings of differential effects of stress and potential circadian phase-specificity highlights the complex interplay between the specific conditions and circadian phase. Thus, specific recording conditions such as circadian time and environmental context may influence state study results such that comparisons across studies are extremely difficult. Altogether, these issues emphasize the importance of considering circadian time and other environmental conditions for not only studies of animal models of depression, but perhaps also clinical investigations.

Concluding remarks

Our data demonstrate that sleep/wakefulness patterning is altered in both male and female rats born to dams exposed to repeated variable PNS. Sleep changes are a hallmark of depression, and so these findings bolster our previous documentation of behavioral changes in PNS rats that mimic those seen in clinical depression. Moreover, our finding that the prevalence of these changes may depend on the circadian phase is in line with the physiological changes observed in patients. Finally, the manifestation of behavioral state changes appears sensitive to acute stress, like other behavioral and physiological processes in clinical, as well as models of, depression. This study, thus, further reinforces the cogency of the PNS rats as an animal model of depressive disorders.

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