Early-life stress alters sleep structure and the excitatory-inhibitory balance in the nucleus accumbens in aged mice

Ting Wang, Hong-Li Wang, Rui Liu, Han Wang, Yue Zhang, Ya-Xin Sun, Yun-Ai Su, Tian-Mei Si, Ji-Tao Li

National Clinical Research Center for Mental Disorders (Peking University Sixth Hospital/Institute of Mental Health) and the Key Laboratory of Mental Health, Ministry of Health (Peking University), Beijing 100191, China.

Abstract

Background: Exposure to adverse experiences in early life may profoundly reshape the neurodevelopmental trajectories of the brain and lead to long-lasting behavioral and neural alterations. One deleterious effect of early-life stress that manifests in later life is sleep disturbance, but this has not been examined in aged mice and the underlying neural mechanisms remain unknown. Considering the important role of the nucleus accumbens (NAc) in the sleep-wake regulation, this study aimed to assess the effects of early-life stress on the sleep behaviors in aged mice and the potential involvement of the NAc in stress-induced sleep abnormalities.

Methods: Twenty aged male C57BL/6 mice (>16 months, $n = 10$ per group) were used in this study. During post-natal days 2 to 9, dams were provided with either sufficient (control) or a limited nesting and bedding materials (stressed). When the mice were 16 to 17 months old, their sleep-wake behaviors were recorded over 24 h using electroencephalogram and electromyelogram. The amount of each sleep-wake stage, mean duration, and stage transition was analyzed. Then, five animals were randomly chosen from each group and were used to measure the expression levels of vesicular glutamate transporter-1 (VGluT1) and vesicular transporters of γ-aminobutyric acid (VGAT) in the NAc using immunohistochemistry. Group comparisons were carried out using Student *t* test or analysis of variances when appropriate.

Results: Compared with the control mice, the early-life stressed aged mice spent less time awake over 24 h (697.97 ± 77.47 min *vs.* 631.33 ± 34.73 min, $t_{17} = 2.376$, $P = 0.030$), accordingly, non-rapid eye movement sleep time was increased (667.37 ± 62.07 min *vs.* 723.54 ± 39.21 min, $t_{17} = 2.326$, $P = 0.033$) and mean duration of rapid eye movement sleep was prolonged (73.00 ± 8.98 min *vs.* 89.39 ± 12.69 min, $t_{17} = 3.277$, $P = 0.004$). Meanwhile, we observed decreased VGluT1/VGAT ratios in the NAc in the stressed group ($F_{(1, 16)} = 81.04$, $P < 0.001$).

Conclusion: Early adverse experiences disrupt sleep behaviors in aged mice, which might be associated with the excitatory-inhibitory imbalance in the NAc.

Keywords: Early-life stress; Sleep; Aging; Nucleus accumbens; Mice

Introduction

Stressful experiences in the early post-natal period are associated with a wide range of negative consequences in later life, such as an increased risk of psychiatric disorders.[1,2] Recent evidence indicates that sleep disturbance is one of the long-term effects of early-life stress[3] based on consistent association between childhood adversities and poor sleep quality in adult humans[4-6] and rodents.[7,8] Given that sleep disturbance causes a severe burden on individuals and society and constitutes an early predictor of the future development of emotional and cognitive disorders,[9,10] understanding how early-life stress influences sleep behaviors has significant clinical importance.

Sleep disturbance is a common complaint in elderly people, affecting approximately 40% of the population.[11,12] Normal aging influences the sleep architecture in several ways.[13] In rodents, it has been reported that aged mice spend less time being awake, especially during the active phase, compared with the spent by young adults.[14,15] It remains unclear whether and how this age-specific sleep pattern interacts with early-life stress exposure. Considering that sleep disturbances in elderly people are related to several risk factors including depressed mood,[16] which is strongly associated with early-life stress exposure,[1] it is possible that sleep disturbances in elderly individuals may be partially attributed to early-life stress exposure. This was confirmed in one study showing that early-life

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.1097/CM9.0000000000000279

Correspondence to: Dr. Tian-Mei Si, National Clinical Research Center for Mental Disorders (Peking University Sixth Hospital/Institute of Mental Health) and the Key Laboratory of Mental Health, Ministry of Health (Peking University), Beijing 100191, China
E-Mail: si.tian-mei@163.com

Chinese Medical Journal 2019;132(13)

Received: 25-01-2019 **Edited by:** Li-Min Chen

emotional abuse is significantly associated with more subjective sleep complaints in elderly people.[17] Based on these clinical observations, animal studies are warranted to be carried out to establish the causal relationships between early-life stress and sleep disturbances in the elderly and to investigate the underlying neural mechanisms so as to develop potential intervention strategies.

The nucleus accumbens (NAc) has been increasingly recognized as an integral part of the sleep-wake regulatory network.[18-20] Selective Nac lesions may increase wakefulness and reduce the mean duration of non-rapid eye movement (NREM) sleep.[21] Critically, the excitatory/inhibitory (E/I) balance of Nac neurons has been found to be sensitive to sleep alterations.[22] While early-life stress reduces γ-aminobutyric acid $(GABA)_A$ receptor-mediated miniature inhibitory post-synaptic currents in the Nac in adult animals,[23] it remains unknown whether and how the Nac E/I balance in the aged mice is affected by early-life stress.

The goal of this study was to examine the effects of early-life stress on sleep-wake behaviors and the Nac E/I balance in aged mice. We adopted the limited nesting and bedding material paradigm, which leads to fragmented and unpredictable maternal care by placing the new mother and pups in a barren living environment and mimics the abnormal maternal care and maternal neglect/abuse frequently observed in human society (eg, when human caregivers are depressed, severely stressed).[24-26] Sleep-wake behaviors were measured by the electroencephalogram (EEG) and electromyelogram (EMG). To characterize the E/I balance in the Nac, we examined the expression levels of the vesicular transporters of glutamate and GABA in the presynaptic terminals, that is, vesicular glutamate transporter-1 (VGluT1) and vesicular GABA transporter (VGAT). These transporters are responsible for the uptake of the corresponding neurotransmitter into synaptic vesicles for subsequent release into the synaptic cleft,[27] and can, therefore, inform us of E/I neurotransmission at the synaptic level.[28]

Methods

Animals

Adult male and female C57BL/6N mice were obtained from Vital River Laboratories (Beijing, China) for breeding. After habituation, each female was housed with one male for 2 weeks and then singly-housed. The pregnant females were monitored daily for pup delivery, and the day of parturition was defined as post-natal day 0 (P0). The male offspring were used for the experiments.

All mice were housed under a 12-h light/dark cycle (lights on at 8 AM) and at a constant temperature (23 ± 1°C) in a humidity-controlled vivarium and had unlimited access to both food and water. This study was carried out in accordance with the recommendations of the National Institute of Health's Guide for the Use and Care of Laboratory Animals. The protocol was approved by the Peking University Committee on Animal Care and Use.

Early post-natal stress paradigm

The limited nesting and bedding material paradigm was used as an early-life stressor and was carried out as previously described.[25,29] Briefly, on the morning of P2, the pups were weighed, and the litters were culled to six to eight pups with an equal number of males and females whenever possible. The control dams were provided with a sufficient amount of nesting material (2 squares [4.8 g] of nestlets; Indulab, Gams, Switzerland) and 500 mL of standard sawdust bedding. In the "stressed" cages, the dams were provided with a limited quantity of nesting material (1/2 square [1.2 g] of nestlets) placed on a fine-gauge aluminum mesh platform (McNichols, Tampa, FL, USA), and the floor was covered with 200 mL of corncob bedding to allow the collection of droppings. The stress procedure ended on the morning of P9. The male offspring were weaned at P28 and group-housed with 3 to 4 animals per cage. Ten animals from each group were randomly selected (using the random function in the Excel, Microsoft, USA) for the following experiments.

Surgical procedure

Male C57BL/6N mice (16–17 months old) were anesthetized by an intra-peritoneal (i.p.) injection of pentobarbital (100 mg/kg) and implanted with EEG and EMG electrodes for polysomnographic recordings. Briefly, for monitoring the EEG signals, two stainless steel EEG recording screws were positioned 1 mm anterior to bregma or lambda, both 1.5 mm lateral to the midline. A reference screw was inserted into the skull on top of the cerebellum. The electrodes were fixed to the skull using dental cement. The bilateral EMG stainless steel wires were inserted into the trapezius (neck) muscles to record skeletal activity. The incision site was closed with non-dissolvable silk sutures and treated with a topical antibiotic (erythromycin ointment) on the first day after the surgery. The mice were given 10 days to recover from surgery before they were acclimated to the recording cables.

EEG recording and analysis

After a 10-day recovery period, the mice were placed in individual sleep-recording chambers (Blackrock Microsystems, Salt Lake City, USA) for acclimatization (once per day for 3 days). The animals were allowed relatively unrestricted movement in the recording cages, which were kept under the same lighting and temperature conditions as the cages in the pre-operative period. Flexible cables that were mounted to fixed commutators were attached to the electrode pedestals. EEG/EMG signals were then monitored for a period of 24 h, beginning at 8:00 AM. One animal in the stressed group was excluded from the data analysis due to a poor connection with the recording cable.

The EEG/EMG signals were amplified and filtered (EEG: 0.5–30 Hz; EMG: 20–200 Hz), digitized at a sampling rate of 500 Hz and analyzed using SleepSign software (Kissei Comtec Co., Nagano, Japan) [Figure 1A]. The vigilance states were divided into 4-s epochs and each epoch was classified as wakefulness, NREM sleep, or REM sleep [Figure 1B]. Wakefulness was identified by high frequency,

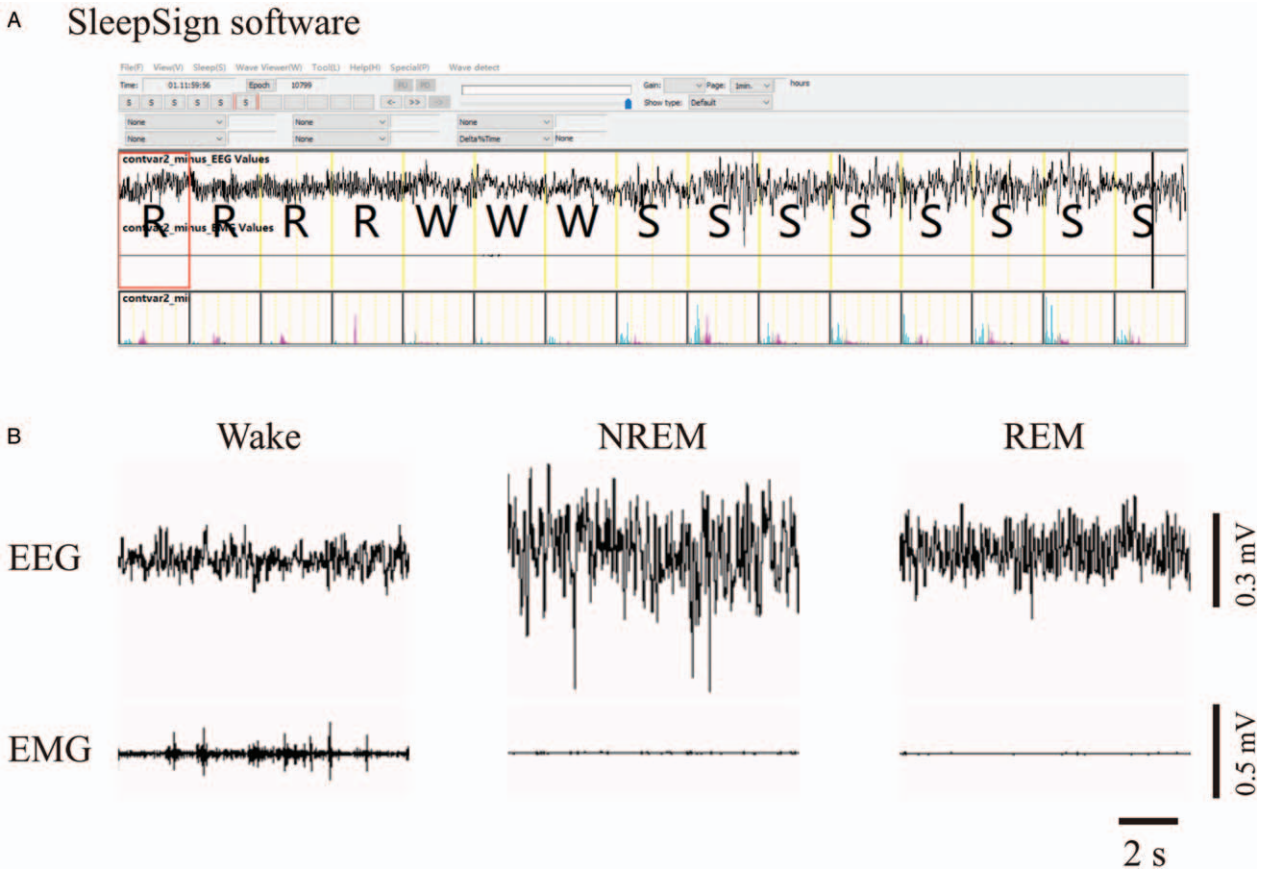

Figure 1: The sleep bioassay system for aged mice. (A) SleepSign software analysis window. (B) Typical examples of EEG and EMG over 10 s during wakefulness, NREM, or REM sleep in an aged mouse. EEG: Electroencephalogram; EMG: Electromyogram; NREM: Non-rapid eye movement; REM: Rapid eye movement.

desynchronized EEG, and frequent EMG activities. The NREM sleep was identified by the dominant presence of high-amplitude, low frequency (0.5–4.0 Hz) EEG activities in the absence of motor activity. The REM sleep was identified by θ waves (4–9 Hz) of consistent low amplitude on the EEG recording accompanied by muscle atonia. Vigilance states assigned by SleepSign (Kissei Comtec) were examined visually and corrected manually if necessary.

Immunohistochemistry

Five animals were randomly chosen (using the random function in the Excel) from each group for immunohistochemical analysis. The animals were deeply anesthetized with pentobarbital sodium (50 mg/kg, i.p.) and then perfused through the left ventricle of the heart with 0.9% saline followed by 4% paraformaldehyde. The brains were removed and post-fixed in 4% paraformaldehyde, and then placed in 30% sucrose in phosphate-buffered saline (PBS) for 3 days at 4°C. The brains were then quenched in N-hexane at a temperature of −60°C for 20 s, and stored in a −80°C freezer.

Serial coronal brain sections were cut through the NAc (Bregma 1.70–0.86 mm) using a cryostat (Leica, Wetzlar, Germany) at 30 μm thickness and 180 μm intervals. A total of three slices per animal for each protein were randomly chosen and the same slice locations were used for each animal to make the results across animals comparable. Immunohistochemistry was performed on free-floating sections. In brief, sections were rinsed in PBS, incubated in 3% hydrogen peroxide for 10 min followed by 1% normal goat serum for 1.5 h and were labeled with the rabbit anti-VGAT (1:1000, Cat. No. 131013, Synaptic systems, Germany) or rabbit anti-VGluT1 (1:10,000, Cat. No. 135302, Synaptic systems, Germany) at 4°C overnight. The next day, sections were rinsed and treated with a biotinylated goat anti-rabbit secondary antibody (Zhongshan Golden Bridge Biotechnology, Beijing, China) at room temperature for 1.5 h. After rinsing, sections were stained with the 3,3′-Diaminobenzidine Horseradish Peroxidase Color Development Kit (Zhongshan Golden Bridge), mounted onto slides and coverslipped.

During image analysis, brain sections were randomly coded by investigators blind to the experimental conditions. Images from three sections per animal were acquired at 100× using an Olympus VS120 microscope, and digital photomicrographs were analyzed by the NIH ImageJ software. As the NAc is anatomically divided into the core and shell[30] and the two sub-regions have been associated with different functions in sleep regulation,[19] we examined their expressions levels and ratios in these sub-regions, respectively. The differences in optical density values of VGluT1 and VGAT between the sub-regions and the anterior part of the anterior commissure (background) were taken as the relative levels of VGluT1 and VGAT.

Statistical analysis

SPSS 24.0 (SPSS, Chicago, IL, USA) and GraphPad Prism 5.0 (GraphPad Software, San Diego, CA, USA) were used to perform statistical analysis. Sleep-wake indices averaged over 24 h were compared between the stressed and control groups using Student *t* test. Sleep-wake indices averaged over the light or dark phase or every 3 h were analyzed by repeated-measures analysis of variance (ANOVA), with the group as the between-subject factor and the phase/time as the within-subject factor, followed by Bonferroni *post hoc* test when appropriate. Two-way ANOVA was used to analyze the expression levels of VGluT1 and VGAT and their ratios (with the group and the NAc sub-region as the factors). Data are reported as mean ± standard error of the mean. The significance level for all statistical tests was $P < 0.05$.

Results

Early-life stress decreased wakefulness and increased NREM sleep in aged mice

We investigated the total time spent awake or in NREM and REM sleep using EEG/EMG recordings in aged mice exposed to either a standard or impoverished childhood environment (P2–9).

We found that the early-life stressed aged mice spent less time awake over 24 h than that spent by the control mice ($t_{17} = 2.376$, $P = 0.030$) [Figure 2A]. Repeated measures ANOVA for the amount of time spent awake during the light and dark phases showed significant main effects of group ($F_{(1,\ 17)} = 5.576$, $P = 0.030$) and phase ($F_{(1,\ 17)} = 286.6$, $P < 0.001$), but there was no group × phase interaction ($F_{(1,\ 17)} = 1.936$, $P = 0.182$). To identify the exact time periods that contributed to the group differences, planned comparisons were performed for each phase, and they revealed that the stress-induced reduction in wakefulness reached significance during the dark phase but not the light phase (dark: $P < 0.05$; light: $P > 0.05$, adjusted with Bonferroni) [Figure 2B]. A further examination of the wakeful time every 3 h revealed that early-life stressed aged mice were significantly less wakeful in the first 3 h of the dark phase ($P < 0.01$, adjusted with Bonferroni) [Figure 2C].

An increased amount of time spent in NREM sleep over 24 h accompanied the reduced wakefulness in the stressed mice ($t_{17} = 2.326$, $P = 0.033$) [Figure 2D]. Similarly, two-way repeated-measures ANOVA revealed significant main effects of group ($F_{(1,\ 17)} = 5.409$, $P = 0.033$) and phase ($F_{(1,\ 17)} = 203.1$, $P < 0.001$) without a group × phase interaction ($F_{(1,\ 17)} = 1.679$, $P = 0.212$). The increase in NREM sleep was significant in the dark phase but not in the light phase (dark: $P < 0.05$; light: $P > 0.05$, adjusted with Bonferroni) [Figure 2E]. NREM sleep was also significantly increased in the first 3 h of the dark phase ($P < 0.01$, adjusted with Bonferroni) [Figure 2F].

REM sleep time was not altered by early-life stress when averaged over 24 h ($t_{17} = 1.332$, $P = 0.200$) [Figure 2G], over either the light or dark phase (Ps > 0.05) [Figure 2H], or every 3 h (Ps > 0.05) [Figure 2I].

Early-life stress increased the mean REM duration in aged mice

Next, we examined the sleep-wake behaviors by breaking down the total time per state into the number of episodes and the mean duration per episode. We found no significant group differences in the number of episodes of each of the three states over 24 h or during each phase [Figure 3A–F]. The mean durations of wakefulness and NREM sleep were also comparable between the stressed and control mice [Figure 3G, 3H, 3J, and 3K]. Nevertheless, the stressed mice showed a significantly longer mean duration of REM sleep than that of the control mice when averaged over 24 h ($t_{17} = 3.277$, $P = 0.004$) [Figure 3I]. Two-way repeated measures ANOVA revealed significant main effects of group ($F_{(1,\ 17)} = 10.74$, $P = 0.004$) and phase ($F_{(1,\ 17)} = 6.310$, $P = 0.022$) and no group × phase interaction ($F_{(1,\ 17)} = 1.080$, $P = 0.313$). A further examination of the REM mean duration in each phase showed significant increases in the dark but not in the light phase (dark: $P < 0.01$; light: $P > 0.05$; adjusted with Bonferroni) [Figure 3L].

Early-life stress did not alter stage transitions in aged mice

We also examined the transitions between each state and found no significant group differences for the number of each transition or the total number of transitions (Ps > 0.082) over 24 h [Figure 4A] or in either the light or dark phase [Figure 4B and 4C].

Early-life stress decreased the E/I ratio in the NAc

Finally, to understand the mechanism underlying the changes in the sleep-wake behaviors, we examined the E/I balance in the NAc core and shell by measuring the expression levels of VGluT1 and VGAT [Figure 5A].

Two-way ANOVA of VGluT1 immunoreactivity showed a significant main effect of group ($F_{(1,\ 16)} = 34.20$, $P < 0.001$) and no group × sub-region interaction ($F_{(1,\ 16)} = 0.004$, $P = 0.951$), indicating that early-life stress downregulated the expression level of VGluT1 in the NAc, irrespective of the sub-region [Figure 5B and 5D]. For VGAT immunoreactivity [Figure 5C and 5E], early-life stress significantly increased the VGAT expression levels throughout the NAc (main effect of group: $F_{(1,\ 16)} = 26.78$, $P < 0.001$). Again, two-way ANOVA revealed the absence of a group × sub-region interaction ($F_{(1,\ 16)} = 0.667$, $P = 0.426$). The VGluT1/VGAT ratio was significantly reduced in the mice exposed to early-life stress (main effect of group: $F_{(1,\ 16)} = 81.04$, $P < 0.001$) [Figure 5F], and the reduction was present in both of the sub-regions as suggested by the lack of a group × sub-region interaction ($F_{(1,\ 16)} = 0.124$, $P = 0.730$).

Discussion

In this study, we examined the effects of early-life stress on the sleep-wake behaviors and the NAc E/I balance in aged mice. We found that stressed aged mice showed abnormal sleep behaviors, including decreased wakefulness, an increased amount of NREM sleep, and an increased duration of REM sleep per episode, especially in the active

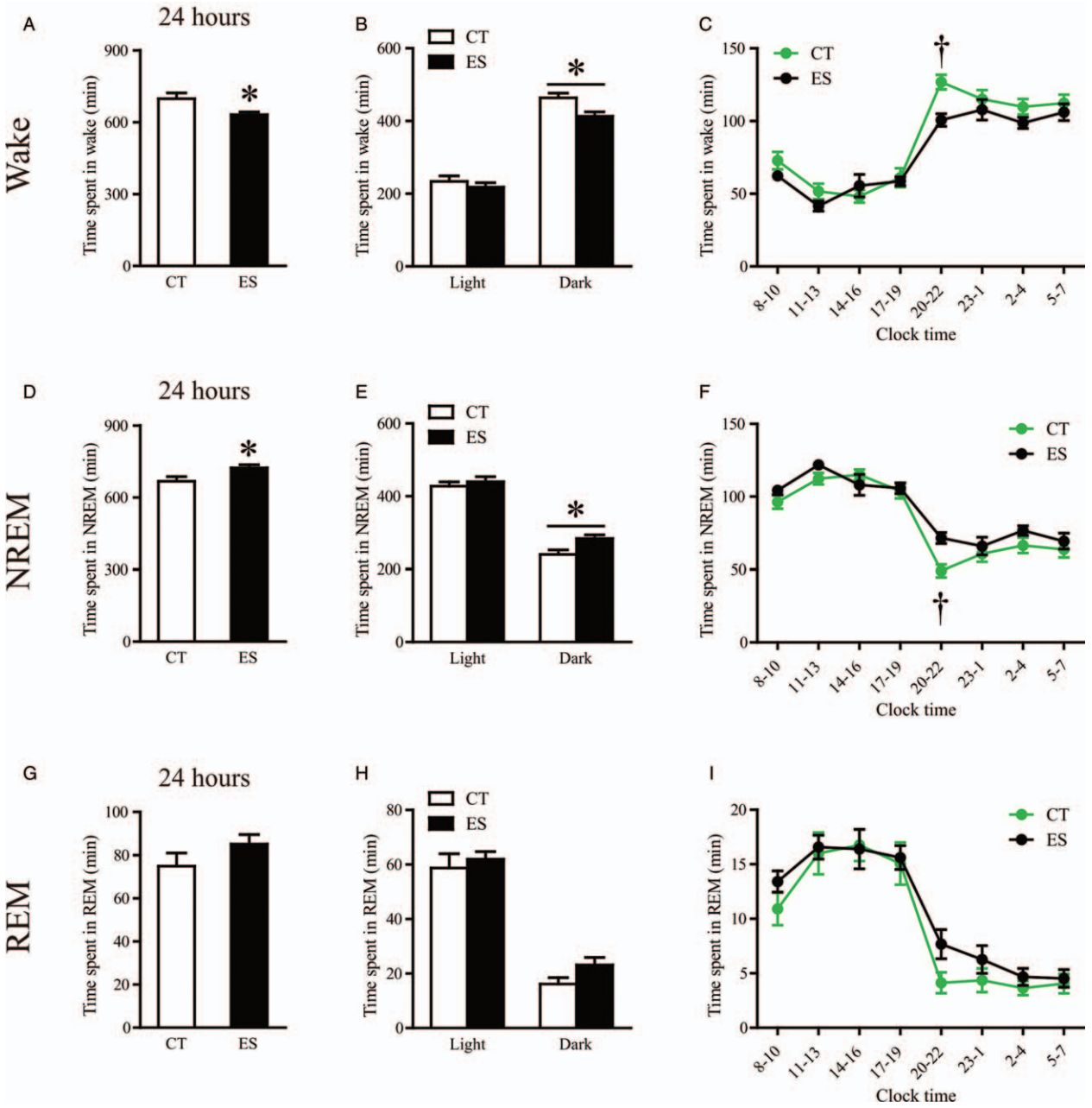

Figure 2: Early-life stress decreased wakefulness and increased NREM sleep in aged mice. (A and B) Early-life stress reduced the amount of wakeful time in aged mice over 24 h (A), especially during the dark phase (B). (C) The amount of time spent awake every 3 h across a light/dark cycle. (D and E) Early-life stress increased the amount of time spent in NREM sleep in aged mice over the 24 h (D) and during the dark phase (E). (F) The amount of time spent in NREM sleep every 3 h across a light/dark cycle. (G–I) Early-life stress did not significantly affect the amount of time spent in REM sleep over 24 h (G), during the light or dark phase (H) or in 3-h bins across a light/dark cycle (I). $^{*}P < 0.05$, $^{\dagger}P < 0.01$. The data are presented as the mean ± standard error. ($n = 9$–10 per group). CT: Control; ES: Early-life stress; NREM: Non-rapid eye movement; REM: Rapid eye movement.

period, compared with those of control mice. These behavioral abnormalities were accompanied by a decreased E/I ratio in the NAc that resulted from decreased VGluT1 expression and increased VGAT expression in the stressed aged mice. These findings extend previous research about the effects of early-life stress on sleep in adults to elderly rodents and suggest that stress-induced sleep alterations in the elderly may be associated with abnormal NAc activity.

Our study provided animal evidence that exposure to early-life stress impairs sleep-wake behaviors in aged individuals. One study investigating this phenomenon in elderly humans found that early parental emotional abuse was associated with increased subjective sleep complaints.[17] Using EEG/EMG recordings, we were able to demonstrate that stress-induced sleep alterations in aged mice are characterized by increased NREM time and REM duration per episode (especially in the active period). Such a behavioral profile seems to differ from sleep alterations in adult animals exposed to maternal separation, which often exhibit sleep alterations in the inactive period.[7,8] Future studies are warranted to investigate whether these different effects result from differences in age or from the early-life

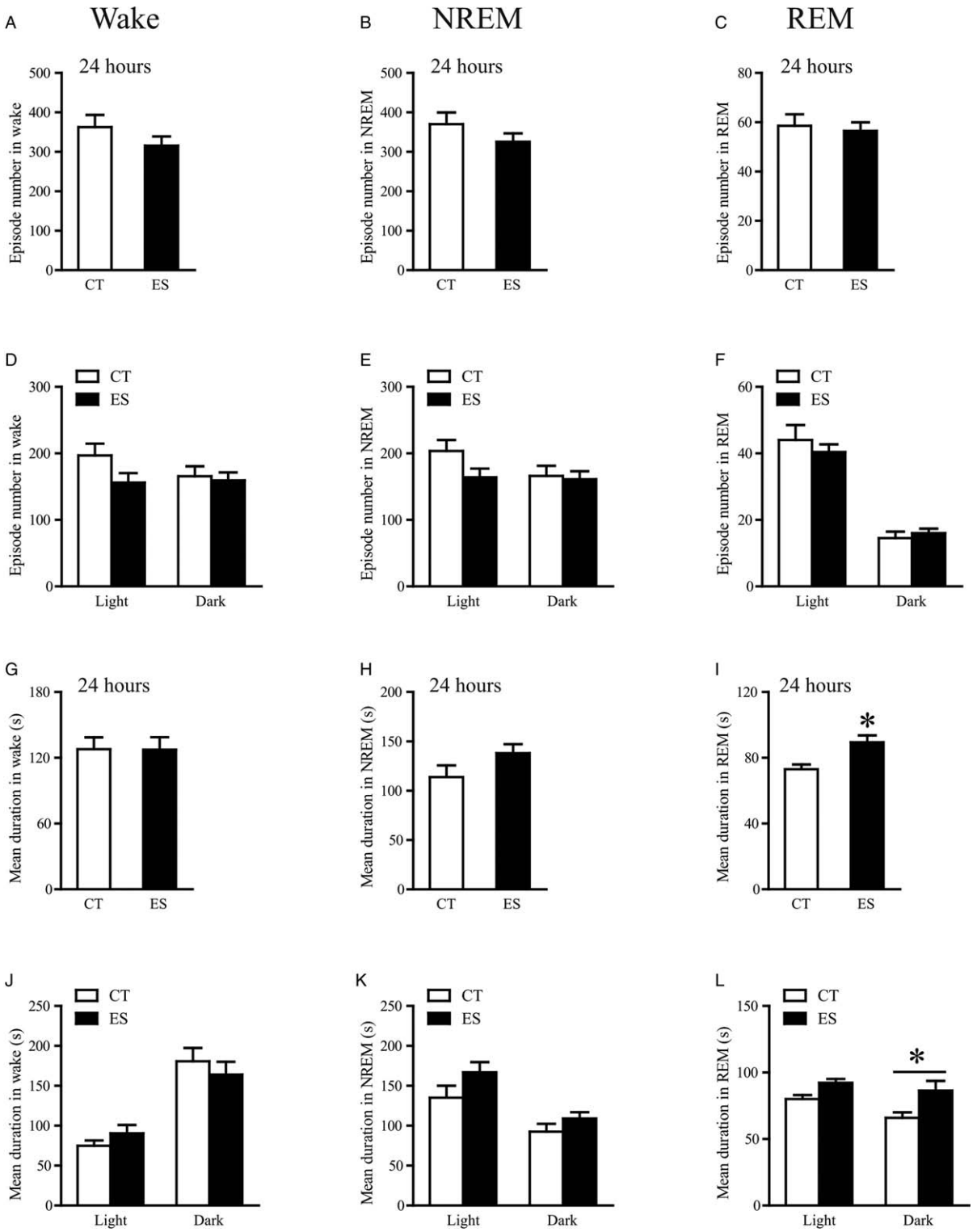

Figure 3: Early-life stress increased the mean REM duration in aged mice. (A–C) The number of episodes of wakefulness (A), NREM sleep (B), and REM sleep (C) over 24 h was not significantly affected by early-life stress. (D–F) Early-life stress did not alter the number of episodes of wakefulness (D), NREM sleep (E), or REM sleep (F) during the light or dark phase in aged mice. (G–L) Early-life stress increased the mean duration of REM sleep per episode (I and L), but not wakefulness (G and J) and NREM sleep (H and K), over 24 h and during the light and dark phases. $^{*}P < 0.01$. The data are presented as the mean ± standard error ($n = 9$–10 per group). CT: Control; ES: Early-life stress; NREM: Non-rapid eye movement; REM: Rapid eye movement.

stress paradigm. Aging influences several aspects of sleep,[13] including decreasing wakefulness, increasing NREM sleep, and inducing signs of sleep fragmentation (eg, increased episodes of wakefulness and NREM and increased transitions between states).[14,15] Our findings of an increased amount of sleep without changes in the number of episodes suggest that early-life stress may exacerbate some aspects of the effects of aging on sleep-wake behaviors.

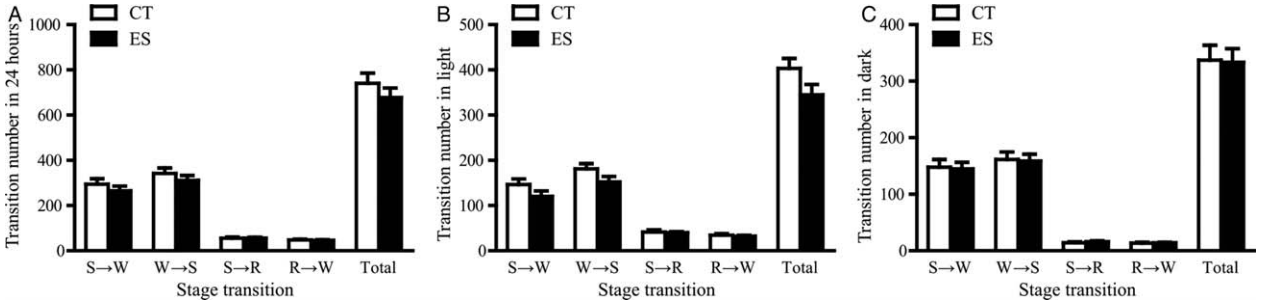

Figure 4: Early-life stress did not affect sleep stage transition in aged mice. Early-life stress did not alter stage transitions over 24 h (A) or during the light phase (B) or the dark phase (C) in aged mice. The data are presented as the mean ± standard error ($n = 9$–10 per group). CT: Control; ES: Early-life stress; R: Rapid eye movement; S: Non-rapid eye movement; W: Wake.

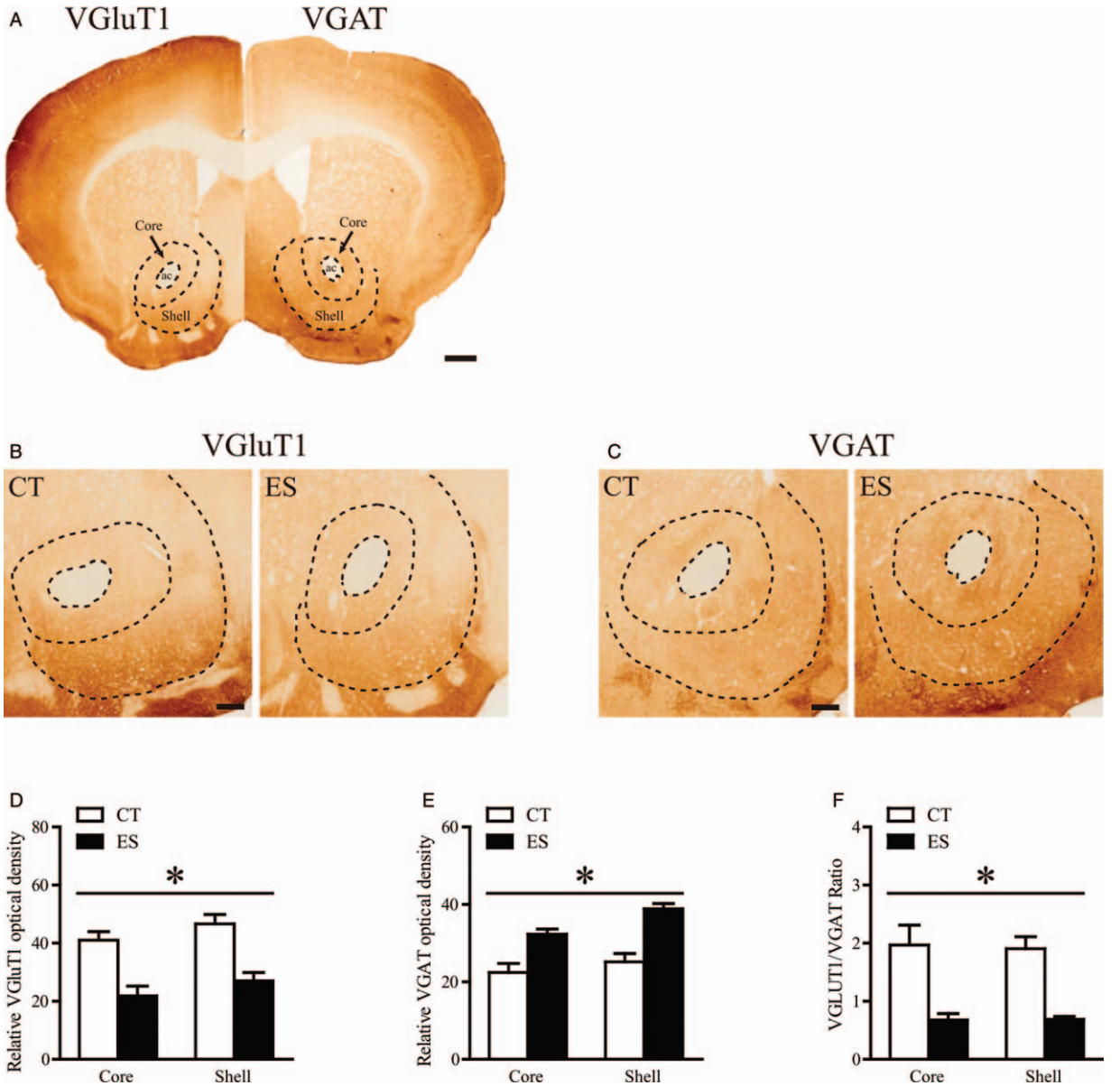

Figure 5: Early-life stress decreased the excitatory/inhibitory ratio in the NAc. (A) A schematic of the two sub-regions of NAc, the core, and shell, used for immunohistochemistry analyses and representative sections immunostained for VGluT1 (left) and VGAT (right). Scale bar = 500 μm. (B and C) Representative immunoreactivity of VGluT1 and VGAT in the CT and ES groups in photographs of 30 μm coronal sections of the two sub-regions of the NAc. Scale bar = 200 μm. (D–F) The relative optical density of VGluT1 (D) and VGAT (E) and their ratio (F) in both groups. $^{*}P < 0.001$, indicating the significance of the main effect of group. The data are presented as the mean ± standard error ($n = 5$ per group). CT: Control; ES: Early-life stress; NAc: nucleus accumbens; VGAT: Vesicular GABA transporter; VGluT1: Vesicular glutamate transporter-1.

The NAc plays important roles in many neurobiological processes, such as reward[31] and addiction.[32] Accumulating evidence has unveiled the mechanisms underlying its involvement in the regulation of sleep-wake behaviors by manipulating distinct neuronal populations via chemogenetic or optogenetic techniques. For instance, the direct stimulation of NAc neurons expressing adenosine A_{2A} receptors/dopamine D_2 receptors induces NREM sleep.[19] Activating NAc neurons expressing dopamine D_1 receptors increases wakefulness, whereas silencing these neurons suppresses arousal.[20] Here, we found that early-life stress decreases the E/I ratio in both the core and shell of the NAc in aged mice. We speculate that the decreased E/I ratio combined with increased sleep behaviors may involve a reduction in D1R-expressing neuronal activity in stressed aged mice. Future studies are invited to examine the E/I balance in distinct populations of NAc neurons to verify this possibility.

The stress-induced reduction of the E/I ratio may result from decreased excitatory inputs (indexed by decreased VGluT1 expression) and increased inhibitory inputs (indexed by increased VGAT expression) on NAc principle neurons. Our previous studies have consistently shown that the early-life stress paradigm simplifies dendritic structural plasticity in the hippocampus and prefrontal cortex in adult mice,[26,33-35] two cortical regions projecting excitatory inputs to the NAc.[22] It is, therefore, possible that the decreased VGluT1 expression in the NAc that was observed in our study may originate from the reduction of cortical excitatory signals. On the other hand, early-life stress has been found to decrease the amplitude and frequency of $GABA_A$ receptor-mediated miniature inhibitory post-synaptic currents in NAc core medium spiny neurons,[23] although the effects on aged mice have not been examined. While this may seem to be at odds with our observation of increased VGAT expression, these neural results are partially in line with the seemingly age-related effects of early-life stress on sleep behaviors (decreased sleep time in the inactive period in adults *vs.* increased sleep time in aged mice). It would be interesting for future studies to determine whether the effects of early-life stress on local NAc inhibition depend on age periods.

The current study is not without limitations. First, the ratio of the VGluT1 and VGAT expression levels is a relatively indirect measure to reflect the neural E/I balance. Future studies with electrophysiological measures should be carried out to validate the E/I imbalance in the NAc induced by early-life stress. Second, our observations of stress-induced changes in sleep behaviors and the VGluT1/VGAT ratio in the NAc are correlational. To examine whether the NAc is causally involved in stress-induced sleep changes, future studies could investigate whether and how sleep behaviors would change following the chemogenetic or optogenetic manipulation of the NAc neurons in post-natally stressed mice.

In conclusion, exposure to early-life stress increases sleep and decreases the E/I ratio in the NAc in aged mice. These results provide preclinical evidence that early adverse experiences confer lifelong impacts on sleep behaviors, that may be associated with an excitatory-inhibitory imbalance in the NAc. Future studies should be conducted to investigate the causal involvement of the NAc in sleep alterations and to develop putative interventions targeting the elderly population.

Acknowledgements

The authors thank Prof. Jie Shi and Mr. Li-Qun Zhang (Peking University) for the use of the sleep-recording system and software, Prof. Zhi-Li Huang (Fudan University) and Dr. Xiang-Yu Cui (Peking University) for their assistance of sleep data analysis.

Funding

This work was supported by grants from the National Key Basic Research Program of China (973 program, No. 2015CB856401), the National Natural Science Foundation of China (Nos. 81630031, 81571321, and 81571312), the Peking University Medicine Seed Fund for Interdisciplinary Research (No. BMU2017MX021), and the Beijing Brain Project (No. Z171100000117016).

Conflicts of interest

None.

References

1. Kessler RC, McLaughlin KA, Green JG, Gruber MJ, Sampson NA, Zaslavsky AM, *et al*. Childhood adversities and adult psychopathology in the WHO World Mental Health Surveys. Br J Psychiatry 2010;197:378–385. doi: 10.1192/bjp.bp.110.080499.
2. Schafer I, Fisher HL. Childhood trauma and psychosis – what is the evidence? Dialogues Clin Neurosci 2011;13:360–365.
3. Palagini L, Drake CL, Gehrman P, Meerlo P, Riemann D. Early-life origin of adult insomnia: does prenatal-early-life stress play a role? Sleep Med 2015;16:446–456. doi: 10.1016/j.sleep.2014.10.013.
4. Chapman DP, Wheaton AG, Anda RF, Croft JB, Edwards VJ, Liu Y, *et al*. Adverse childhood experiences and sleep disturbances in adults. Sleep Med 2011;12:773–779. doi: 10.1016/j.sleep.2011.03.013.
5. Koskenvuo K, Hublin C, Partinen M, Paunio T, Koskenvuo M. Childhood adversities and quality of sleep in adulthood: a population-based study of 26,000 Finns. Sleep Med 2010;11:17–22. doi: 10.1016/j.sleep.2009.03.010.
6. Schafer V, Bader K. Relationship between early-life stress load and sleep in psychiatric outpatients: a sleep diary and actigraphy study. Stress Health 2013;29:177–189. doi: 10.1002/smi.2438.
7. Feng P, Hu Y, Vurbic D, Guo Y. Maternal stress induces adult reduced REM sleep and melatonin level. Dev Neurobiol 2012;72:677–687. doi: 10.1002/dneu.20961.
8. Mrdalj J, Pallesen S, Milde AM, Jellestad FK, Murison R, Ursin R, *et al*. Early and later life stress alter brain activity and sleep in rats. PloS One 2013;8:e69923. doi: 10.1371/journal.pone.0069923.
9. Koren D, Arnon I, Lavie P, Klein E. Sleep complaints as early predictors of posttraumatic stress disorder: a 1-year prospective study of injured survivors of motor vehicle accidents. Am J Psychiatry 2002;159:855–857. doi: 10.1176/appi.ajp.159.5.855.
10. Neckelmann D, Mykletun A, Dahl AA. Chronic insomnia as a risk factor for developing anxiety and depression. Sleep 2007;30:873–880. doi: 10.1093/sleep/30.7.873.
11. Luo J, Zhu G, Zhao Q, Guo Q, Meng H, Hong Z, *et al*. Prevalence and risk factors of poor sleep quality among Chinese elderly in an urban community: results from the Shanghai aging study. PloS One 2013;8:e81261. doi: 10.1371/journal.pone.0081261.
12. Smagula SF, Stone KL, Fabio A, Cauley JA. Risk factors for sleep disturbances in older adults: evidence from prospective studies. Sleep Med Rev 2016;25:21–30. doi: 10.1016/j.smrv.2015.01.003.
13. Mander BA, Winer JR, Walker MP. Sleep and human aging. Neuron 2017;94:19–36. doi: 10.1016/j.neuron.2017.02.004.

14. Hasan S, Dauvilliers Y, Mongrain V, Franken P, Tafti M. Age-related changes in sleep in inbred mice are genotype dependent. Neurobiol Aging 2012;33:195.e113–195.126. doi: 10.1016/j.neurobiolaging.2010.05.010.
15. Wimmer ME, Rising J, Galante RJ, Wyner A, Pack AI, Abel T. Aging in mice reduces the ability to sustain sleep/wake states. PloS One 2013;8:e81880. doi: 10.1371/journal.pone.0081880.
16. Smagula SF, Ancoli-Israel S, Barrett-Connor E, Lane NE, Redline S, Stone KL, *et al*. Inflammation, sleep disturbances, and depressed mood among community-dwelling older men. J Psychosom Res 2014;76:368–373. doi: 10.1016/j.jpsychores.2014.02.005.
17. Poon CY, Knight BG. Impact of childhood parental abuse and neglect on sleep problems in old age. J Gerontol B Psychol Sci Soc Sci 2011;66:307–310. doi: 10.1093/geronb/gbr003.
18. Lazarus M, Chen JF, Urade Y, Huang ZL. Role of the basal ganglia in the control of sleep and wakefulness. Curr Opin Neurobiol 2013;23:780–785. doi: 10.1016/j.conb.2013.02.001.
19. Oishi Y, Xu Q, Wang L, Zhang BJ, Takahashi K, Takata Y, *et al*. Slow-wave sleep is controlled by a subset of nucleus accumbens core neurons in mice. Nat Commun 2017;8:734. doi: 10.1038/s41467-017-00781-4.
20. Luo YJ, Li YD, Wang L, Yang SR, Yuan XS, Wang J, *et al*. Nucleus accumbens controls wakefulness by a subpopulation of neurons expressing dopamine D1 receptors. Nat Commun 2018;9:1576. doi: 10.1038/s41467-018-03889-3.
21. Qiu MH, Vetrivelan R, Fuller PM, Lu J. Basal ganglia control of sleep-wake behavior and cortical activation. Eur J Neurosci 2010;31:499–507. doi: 10.1111/j.1460-9568.2009.07062.x.
22. Liu Z, Wang Y, Cai L, Li Y, Chen B, Dong Y, *et al*. Prefrontal cortex to accumbens projections in sleep regulation of reward. J Neurosci 2016;36:7897–7910. doi: 10.1523/JNEUROSCI.0347-16.2016.
23. Mitchell SJ, Maguire EP, Cunningham L, Gunn BG, Linke M, Zechner U, *et al*. Early-life adversity selectively impairs alpha2-GABAA receptor expression in the mouse nucleus accumbens and influences the behavioral effects of cocaine. Neuropharmacology 2018;141:98–112. doi: 10.1016/j.neuropharm.2018.08.021.
24. Walker CD, Bath KG, Joels M, Korosi A, Larauche M, Lucassen PJ, *et al*. Chronic early life stress induced by limited bedding and nesting (LBN) material in rodents: critical considerations of methodology, outcomes and translational potential. Stress 2017;20:421–448. doi: 10.1080/10253890.2017.1343296.
25. Rice CJ, Sandman CA, Lenjavi MR, Baram TZ. A novel mouse model for acute and long-lasting consequences of early life stress. Endocrinology 2008;149:4892–4900. doi: 10.1210/en.2008-0633.
26. Yang XD, Liao XM, Uribe-Marino A, Liu R, Xie XM, Jia J, *et al*. Stress during a critical postnatal period induces region-specific structural abnormalities and dysfunction of the prefrontal cortex via CRF1. Neuropsychopharmacology 2015;40:1203–1215. doi: 10.1038/npp.2014.304.
27. Eiden LE. The vesicular neurotransmitter transporters: current perspectives and future prospects. FASEB 2000;14:2396–2400. doi: 10.1096/fj.00-0817rev.
28. Li JT, Zhao YY, Wang HL, Wang XD, Su YA, Si TM. Long-term effects of neonatal exposure to MK-801 on recognition memory and excitatory-inhibitory balance in rat hippocampus. Neuroscience 2015;308:134–143. doi: 10.1016/j.neuroscience.2015.09.003.
29. Wang XD, Labermaier C, Holsboer F, Wurst W, Deussing JM, Muller MB, *et al*. Early-life stress-induced anxiety-related behavior in adult mice partially requires forebrain corticotropin-releasing hormone receptor 1. Eur J Neurosci 2012;36:2360–2367. doi: 10.1111/j.1460-9568.2012.08148.x.
30. Zahm DS. Functional-anatomical implications of the nucleus accumbens core and shell subterritories. Ann N Y Acad Sci 1999;877:113–128. doi: 10.1111/j.1749-6632.1999.tb09264.x.
31. Russo SJ, Nestler EJ. The brain reward circuitry in mood disorders. Nat Rev Neurosci 2013;14:609–625. doi: 10.1038/nrn3381.
32. Volkow ND, Morales M. The brain on drugs: from reward to addiction. Cell 2015;162:712–725. doi: 10.1016/j.cell.2015.07.046.
33. Li JT, Xie XM, Yu JY, Sun YX, Liao XM, Wang XX, *et al*. Suppressed calbindin levels in hippocampal excitatory neurons mediate stress-induced memory loss. Cell Rep 2017;21:891–900. doi: 10.1016/j.celrep.2017.10.006.
34. Liao XM, Yang XD, Jia J, Li JT, Xie XM, Su YA, *et al*. Blockade of corticotropin-releasing hormone receptor 1 attenuates early-life stress-induced synaptic abnormalities in the neonatal hippocampus. Hippocampus 2014;24:528–540. doi: 10.1002/hipo.22254.
35. Liu R, Yang XD, Liao XM, Xie XM, Su YA, Li JT, *et al*. Early postnatal stress suppresses the developmental trajectory of hippocampal pyramidal neurons: the role of CRHR1. Brain Struct Funct 2016;221:4525–4536. doi: 10.1007/s00429-016-1182-4.

How to cite this article: Wang T, Wang HL, Liu R, Wang H, Zhang Y, Sun YX, Su YA, Si TM, Li JT. Early-life stress alters sleep structure and the excitatory-inhibitory balance in the nucleus accumbens in aged mice. Chin Med J 2019;132:1582–1590. doi: 10.1097/CM9.0000000000000279