Sleep Deprivation Induces Neuroinflammation and Depressive-like Behaviors by Impairing the Regulation of Circadian Clock Genes Expression in Rats

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Abstract

Background: Sleep loss leads to a spectrum of mood disorders such as anxiety, cognitive dysfunction and motor coordination impairment in many individuals. However, the underlying mechanisms are largely unknown.

Methods: In this study, we examined the effects of sleep deprivation (SD) on depression and the mechanism by subjecting rats to a slowly rotating platform for 3 days to mimic the process of sleep loss. Sleep-deprived animals were tested behaviorally for anxiety- and depressive-like behaviors. We further studied the effects of SD on hypothalamic-pituitary-adrenal (HPA) axis activity, and at the end of the experiment, brains were collected to measure the circadian clock genes expression in the hypothalamus, glial cell activation and inflammatory cytokine alterations.

Results: Our results indicated that SD for 3 days resulted in anxiety- and depressive-like behaviors. SD exaggerated cortisol response to HPA axis, significantly altered the mRNA profile of circadian clock genes, and induced neuroinflammation by increasing the expression of glial cell markers, including the microglial marker ionized calcium-binding adapter molecule 1 (Iba1) and the astroglial marker glial fibrillary acidic protein (GFAP). The expression of M1 and M2 microglial markers (Arg-1 and CD206, respectively) and pro-inflammatory cytokines (IL-1β, IL-6 and TNF-α) were increased in the brain.

Conclusion: These results indicated that SD for 3 days induced anxiety- and depression-like behaviors in rats by impairing the regulation of circadian clock genes and inducing neuroinflammation, ultimately resulting in brain injury.

Introduction

Sleep, as a physiological phenomenon, has been found to play important roles in the functioning of the nervous, muscular and immune systems, leading to accentuated growth and rejuvenation(Bass and Takahashi, 2010; Ferguson et al., 2016). A lack of sleep is a common problem in modern society, and it has severe consequences for health and results in related neurological disorders that consume a large fraction of health care resources. Sleep loss has a profound, negative impact on cognition, learning, mood, and diverse aspects of mental health. Despite decades of research, the reasons why sleep loss negatively impacts brain function have remained unclear.

Circadian rhythms, defined as oscillations with a period of 24 h, are a fundamental component of mammalian physiology. Sleep is clearly regulated by the circadian clock, as human sleep patterns follow clear circadian patterns. Mammalian circadian rhythms are regulated by a molecular transcription/translation feedback loop involving transcriptional inhibitory proteins (PER1, PER2, CRY1, and CRY2) and transcriptional activating proteins (BMAL1, CLOCK, and NPAS2) (Ho et al., 2018; Kayaba et al., 2017; Lauretti et al., 2017; Pan and Rickard, 2015). The deletion of the master clock gene BMAL1 abrogates all circadian function, leading to a total loss of the day-night rhythmicity of sleep and massive astrocyte activation and inflammation(Aaron et al., 2005; Li et al., 2013; Ratajczak et al., 2009). Circadian

rhythm impairments occur in many mental disorders such as depression (Bhattacharjee, 2007), manic disorders (Yin et al., 2006;Toh et al., 2001). Patients with major depressive disorder (MDD) presented with disruptions in biological circadian rhythms (Kronfeld-Schor and Einat, 2012).

Circadian rhythm dysfunction has been associated with inflammation in the periphery, but the role of the circadian clock genes in neuroinflammation remains poorly understood. Neuroinflammation is widely regarded as inflammation of the central nervous system, which includes microglia activation and increases in the levels of pro-inflammatory cytokines in the brain, and may lead to cognitive function decline(Aguirre, 2016; Rasch and Born, 2013). Various clinical studies have also documented the occurrence of inflammation caused by sleep loss(Siegel, 2005). Recent studies have provided evidence that both chronic sleep loss and fragmentation can activate glial cell types and influence behavioral and physiological states(Graves, 2003; Ho et al., 2018; Wisor et al., 2008). In addition, the activation of the sympathetic nervous system and HPA axis after sleep disturbance leads to a heightened pro-inflammatory state. However, the exact neuropathological mechanism underlying sleep loss induced neuronal dysfunction is not fully understood.

The present study aimed to investigate whether sleep loss triggers anxiety- and depressive-like behaviors accompanied by the disruption the regulation of circadian clock genes, the activation of inflammatory mediators with reactive astrogliosis and microgliosis in rats. Rats were first subjected to behavioral tests after 3 days of sleep deprivation (SD) to evaluate emotional impairment and were then sacrificed brain collection for further molecular studies. Then, we examined the pathological events associated with neuroinflammation, such as the activation status of microglia and astrocytes as well as the expression of pro-inflammatory cytokines. We also demonstrated the underlying mechanisms of SD-associated neurobehavioral alterations, the effects of SD on the HPA axis activity and the mRNA profile of circadian clock genes in the hypothalamus. Our results demonstrated that the impact of SD on depression highlights the importance of neuroinflammation in adapting to sleep loss. One mechanism through which SD may contribute to depressive symptomatology is by affecting the regulation of circadian clock genes and ultimately causing circadian rhythm dysfunction. These data suggest that SD for 3 days induces anxiety- and depression-like behaviors in rats by dysregulating circadian clock genes expression and inducing microglial proinflammatory cytokines, resulting in brain injury.

Methods

Animal

Animal experiments

Adult male Sprague-Dawley rats (200–220 g) were purchased from Vital River Laboratory Animal Technology Co. (Beijing, China). Animals were maintained under a 12 h light/12 h dark cycle at 22 °C. Food and water were available ad libitum. Temperature was maintained between 19 °C and 21 °C and lights were on at 7:00 A.M. and off at 7:00 P.M. Zeitgeber time (ZT) 0 is here defined as lights on,

corresponding to 7:00 A.M. All animal experiments were approved by the Institutional Animal Care and Use Committee of Beijing Institute of Basic Medical Sciences and performed in accordance with the guidelines.

Open field test

An open field test (OFT) was performed to assess the locomotor activity. Locomotor activity in rats was tested in a quiet open field using a locomotion analyzing system (JLBehv-LAR-1; Shanghai Jiliang Software Technology Co. Ltd). Briefly, rats were placed in the central of the open-field equipment and allowed to freely explore the area for 5 min. The total distances travelled in the open area was recorded and calculated with a video camera link to computer and regarded as a parameter of motor ability. The apparatus was cleaned between each rat, using 70% ethylalcohol.

Tail suspension test

The tail suspension test (TST) in rats was performed as described by our previous reports. In brief, the rats were suspended with a hook by approximately 1 cm from the tip of the tail using adhesive tape. The experimental period was videotaped and the duration of immobility time was observed for 5 min. Immobility was considered as the animal remaining motionless or making only minor, non-escape-related movements.

Elevated plus maze test

Elevated plus maze test (EPMT) was commonly used to assess anxiety-like behavior as previously described. The rats were individually placed in the central zone facing one of the open arms, video camera mounted above the maze connected to a computer was used to monitored and scored the explore behavior, during 5-min experimental period. The percentage of open-arm entries and the proportion of time spent in the open arms were calculated for each animal. The more decreased in open arm activity, the more reflected anxiety behavior.

Sucrose Preference Test

Rats were singly housed to allow individual fluid intake measurements. After 48 h of adaptation to the experimental set up (two bottles of water), the rats had access to sucrose solution in one bottle and tap water for the remainder of the experiment. Water and sucrose solutions were replaced after 24 h and weighed to measure the consumption of sucrose.

Serum collection and tissue preparation

Fresh blood samples from the rat were allowed to stand for 2 h at room temperature, and then centrifuged at 2000 g at 4 °C for 20 min. The serum was transferred into a new tube and stored at -80 °C until use. The serum level of Cortisol and Inflammatory cytokine were measured using an ELISA kit from Applygen Technologies Inc. Assays were performed according to the protocols provided by the manufacturer. After behavioral tests, the rats were anaesthetized and sacrificed by decapitation. The brains were rapidly removed, and the brain tissue was dissected and frozen at -80 °C for future western

blot analysis. The brains used for immunohistochemistry analysis were fixed in 4% formaldehyde immediately after isolation.

Enzyme-linked immunosorbent assay

Serum was collected for enzyme-linked immunosorbent assay (ELISA). For tissue lysate preparation, rat brain tissue was homogenized with cell lysis buffer on ice for 30 min, and then the homogenate was centrifuged at 12000 g for 30 min. The supernatant was aliquoted and stored for further experiments. The levels of Cortisol, TNF-a, IL-1, and IL-6 in serum and brain tissue were determined with the corresponding rat ELISA kits (R&D systems) according to the manufacturer's instructions.

Immunohistochemistry analyses

Rats were re-anesthetized and transcardially perfused with normal saline, followed by 4% paraformaldehyde (PFA) in 0.1 M PBS. The brain was removed and immersed in the same fixative at 4 °C for 24 h and then transferred into 15% sucrose in PBS for 24 h and 30% sucrose in PBS for 24 h and embedded in O.C.T. compound. The embedded tissue was immediately frozen in liquid nitrogen and stored at − 20 °C until use. The coronal sections of the brain frozen sections were cut with a cryostat at 15 μm, and mounted onto glass slides, as previously described. For immunofluorescence staining, brain sections were permeabilized with 0.01% Triton X-100 and 10% normal goat serum in PBS, pH 7.4, for 60 min. Then the sections were stained with primary antibodies against Iba-1 (1:200; microglia marker; rabbit, Proteintech), GFAP (1:500; astrocyte marker, rat, Dako). The sections were then incubated with Alexa Fluor-conjugated secondary antibodies (1:250; Invitrogen). Nuclear counterstaining was performed using Hoechst 33342 (1:1000; Roche).

Hematoxylin and Eosin Staining

Brain tissues of 4–6 rats from each experimental group were fixed in 10% neutral buffered formalin for 48 h, and then embedded in paraffin and routinely sectioned at 3–5um. After xylene de-waxing and graded ethanol debenzolization, the sections were stained with eosin and hematoxylin under microscope (Olympus, Japan).

Western blot analysis

Proteins were extracted in ice-cold RIPA buffer containing protein inhibitors cocktail (Roche). The dissolved proteins were collected after centrifugation at 10,000 °C for 10 min at 4 °C, and the supernatant was then collected. The protein concentration was determined using a Pierce BCA Protein Assay kit (Thermo Scientific). A quantity of 120 μg of total protein was loaded onto a 10 ± 15% SDS-PAGE, electrophoretically transferred to polyvinylidene difluoride membrane (PVDF), and probed with the following primary antibodies: Clock (Santa Cruz), Bmal1 (Santa Cruz), Per1 (Santa Cruz), Per2 (Santa Cruz), Cry1 (Proteintech), Cry2 (Proteintech) and β-actin (Proteintech) was used as an internal control. The membranes were developed using an enhanced chemiluminescence detection system. For densitometry of the protein bands, the optical densities (OD) of the protein bands were quantified using

Quantity One software (Bio-Rad). The relative band densities were calculated as the ratio of the protein band OD relative to the β-actin (internal loading control) band OD from the same sample.

Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from the Hypothalamus of the rat brains using Trizol reagent (Invitrogen, USA). First-stand cDNA of each sample was synthesized using an MLV reverse transcription kit (TAKARA, Japan) according to the manufacturer's instructions. cDNA was used as a template for quantitative real-time PCR using SYBR green master mix (Applied Biosystems, USA). The primers used are as follows: Clock-F: 5'-TGGCTGACACTTTTGAGCAC-3', Clock-R: 5'-GAAGTGTACAAGTCCGCGTC-3'; Bmal1-F: 5'-TTGCCCTAGACTTCGAGCAA-3', Bmal1-R: 5'- CAGGAAGGAAGGCTGGAAGA-3'; Per1-F: 5'-TTGCCCTAGACTTCGAGCAA-3', Per1-R: 5'- CAGGAAGGAAGGCTGGAAGA-3'Per2-F: 5'-TTGCCCTAGACTTCGAGCAA-3', Per2-R: 5'- CAGGAAGGAAGGCTGGAAGA-3' Cry1-F: 5'-TTGCCCTAGACTTCGAGCAA-3', Cry1-R: 5'- CAGGAAGGAAGGCTGGAAGA-3' Cry2-F: 5'-TTGCCCTAGACTTCGAGCAA-3', Cry2-R: 5'- CAGGAAGGAAGGCTGGAAGA-3' β-actin-F: 5'-TTGCCCTAGACTTCGAGCAA-3', β-actin-R: 5'- CAGGAAGGAAGGCTGGAAGA-3'Gene expression was normalized to the mRNA levels of β -actin.

Statistical analysis

The data are represented as the means ± SEM. The statistical analyses were performed by Student's t test or a one- or two-way analysis of variance (ANOVA) with a post hoc Tukey's test using GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, CA). The two-tailed, non-paired Student's t test was used for comparison of two groups. A value of $p < 0.05$ was considered to indicate statistical significance.

Result

1. SD induces anxiety- and depressive-like behaviors in rats

The experiments were performed as shown in Fig. 1A. After 3 days of sleep deprivation, the rats were first subjected to behavioral tests. The open field (OF) and elevated plus-maze (EPM) tests were used to evaluate anxiety-like behaviors in sleep-deprived rats. In the OF test, the total distance traveled were increased but the time spent in the center area was obviously decreased in SD rats (Fig. 1B and C). In the EPM test, there is no difference of total entrance to the open and closed arms were observed between control and SD rat. As shown in Fig. 1D, the SD group rats showed an notably increased time spent in the closed arm compared to the open arm, which indicated that anxiety-like behaviors were triggered due to sleep loss. In addition, during the EPM test, SD animals completely prohibited themselves from entering the open arm and spent less time in the open arm. These results indicate that SD induces anxiety-like behaviors in rats.

Since anxiety is associated with poor performance, alertness and cognitive dysfunction, we further analyzed depressive-like behaviors in the rats by the tail suspension test (TST) and sucrose preference test (SPT). The immobility time of the SD rats in the TST was obviously decreased compared to that of

the control rats (Fig. 1E). Furthermore, we compared the consumption of sucrose in SD rats and found that SD rats consumed less sucrose than control rats did, indicating that SD induces depressive-like behaviors (Fig. 1F). These findings are consistent with those of several other studies in which chronic sleep loss was shown to lead to anxiety- and depressive-like behaviors in rats.

2. Transcriptional changes of circadian expression of molecular-clock-related genes in the hypothalamus after SD

To investigate the connection between the depressive behavior and circadian clock genes, expression of molecular circadian clock genes was examined in the SCN during the period of SD. We first performed qRT-PCR analysis from hypothalamic samples containing the SCN collected every fourth hour around the clock. Normalized mRNA transcript profiles from hypothalamus exhibited oscillations for several circadian related genes. Clock and Bmal1 displayed similar circadian expression, with trough expression at ZT12. Cry1, Per1, Per2, Rev-erbα, and Rorα all showed peak amplitude ranging from ZT 8 to ZT 16. We then addressed the alteration of circadian clock genes during the period of SD. We found that almost all circadian clock genes mRNA was altered, and showed abnormal circadian oscillations compared with those of control (Fig. 2A-H). SD increased the amplitude of Bmal1, Per genes, Cry1 and Cry2 transcript levels to a greater extent at day. The expression level of Clock and Bmal1 was affected at night, however, Per1 and Cry2 was unaffected by night sleep deprivation. The alterations in the transcript profiles of circadian genes could lead to altered homeostasis in the hypothalamus with subsequent consequences on numerous pathological processes after SD. These results indicate that the SD rat have impairments in the regulation of circadian clock genes.

3. SD increases the levels of corticosterone in rats

The hypothalamic-pituitary-adrenal (HPA) axis is an adaptive system with the purpose of maintaining a dynamic equilibrium or homeostasis during stress. Corticosterone is the main stress hormone in rodents and is widely known as a classical regulator of the inflammatory and immune response in the brain and periphery(Aguirre, 2016; Kayaba et al., 2017). To assess the effects of SD on the HPA axis, plasma cortisol levels were measured. In our study, we found that the SD rats showed higher levels of cortisol than the control rats did (Fig. 3A), indicating that SD may be an activation of the HPA axis responses. In addition, cortisol levels in the brain are considered a reliable indicator of anxiety onset and maintenance. To determine the effects of SD on the HPA axis in the central nervous system, we quantified the cortisol levels in the cerebrospinal fluid (CSF). After 3 days of SD, the levels of cortisol were significantly higher than those in control rats (Fig. 3B). We conclude that SD exaggerated cortisol response to a stressor indicative of elevated HPA axis responses, which may contribute to the manifestation of depressive symptoms and to the severity of cognitive impairment.

4. SD increases proinflammatory cytokine levels in rats

The activation of the sympathetic nervous system and HPA axis after SD leads to a heightened pro-inflammatory state. However, the exact neuropathological mechanism underlying SD-induced neuronal dysfunction is not fully understood. Given the significant role of neuroinflammation in mental pathology, we hypothesize that neuroinflammation may provide a mechanism of the pathogenesis of psychological impairments. To test whether SD elicits susceptibility to the inflammatory response by inducing proinflammatory cytokines, we examined the concentrations of the proinflammatory cytokines interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor α (TNF-α) in the serum after 3 days of SD. The concentration of IL-1β increased from 36.0 ± 10.4 pg/mL to 54.1 ± 8.3 pg/mL, the IL-6 concentration increased from 26.0 ± 5.7 pg/mL to 32.1 ± 8.6 pg/mL, and there was slightly change in the TNF-α concentration in the serum compared with that in the controls (Fig. 4A-C). Consistent with previous reports, rats challenged with 3 days of SD tolerated SD-induced inflammation much better than control rats did. We further investigated the inflammatory response in the brain after 3 days of SD by evaluating the CSF. The mean IL-1β concentration in the CSF increased from 30.2 ± 12.8 pg/ml to 43.6.8 ± 9.4 pg/ml, and the IL-6 concentration increased from 28.8 ± 9.3 pg/ml to 37.8 ± 7.4 pg/ml. The TNF-a concentration slightly increased from 19.1 ± 6.3 pg/ml to 21.8 ± 7.4 pg/ml. These results indicate that SD induced an inflammatory response and brain injury in rats (Fig. 4D-F).

5. SD-induced inflammatory responses impair the blood-brain barrier in rats

Neuroinflammation is a consequence of the structural and functional disruption of the blood-brain barrier (BBB), which is an early and prominent feature of CNS inflammation. It has been previously reported that circadian rhythm genes controlled by circadian rhythm contribute to the induction of inflammation(Feinstein et al., 2016; Kumar and Chanana, 2014; Manchanda et al., 2018). We hypothesize that SD can affect circadian clock gene expression to disrupt homeostasis in the brain. To further investigate the potential mechanism of inflammation after SD, we assessed whether SD can impair the integrity of the BBB. We first observed morphological changes in the brain caused by SD. Brain sections from control animals showed a normal arrangement and structure of neurons and cerebral capillaries. However, sections from SD brains showed dilatation of the blood vessels and inflammatory invasion, indicating morphological changes in BBB integrity (Fig. 5A). Tight junctions maintain the function of the BBB, and we observed the expression of tight junction proteins using western blotting. We further evaluated the molecular changes in the tight junction proteins ZO-1 and claudin-5; there were marked changes in the expression of these proteins in the rats after 3 days of SD (Fig. 5B). In addition, the expression of aquaporin-4 (AQP-4), a glial membrane water channel in the brain, was increased by nearly 2-fold in the brain, indicating the onset of brain injury (Fig. 5C). These results suggest that SD-induced inflammation accelerates brain injury by disrupting tight junctions and impairing BBB integrity in rats.

6. SD induces astrogliosis and microgliosis in rats

Glial cells, which include astrocytes, microglia, and oligodendrocytes, have been found to play key roles in inflammation and neurological disease(Bellesi et al., 2017; Feinstein et al., 2016). To determine the relationship between inflammation and glial cell activation during SD, we analyzed the immunoreactivity of astrocyte and microglial cell activation markers after SD. The activation of astrocytes was first evaluated by immunostaining for the astrocyte marker glial fibrillary acidic protein (GFAP). We observed changes in GFAP immunostaining and protein levels in the brain. Immunohistochemical staining demonstrated astrocyte activation, with altered morphology and increased the expression of GFAP (Fig. 6A-B). Then, we measured microglial activation by staining for the microglial marker ionized calcium-binding adapter molecule 1 (Iba-1) as a measurement of microglial density. Immunohistochemical staining for Iba-1 showed that SD induced microglial activation by increasing the number of Iba-1-positive cells in the rat cortex and hippocampus compared to that in the control animals (Fig. 6C-D). To further assess whether the inflammatory effect of SD is due to the inhibition of the polarization of microglia from the proinflammatory M1 phenotype to the anti-inflammatory M2 phenotype, we analyzed the expression of the M2 markers arginase 1 (Arg-1) and Ym-1. SD reduced the expression of Arg-1 and CD206 in the brain, and the effects were statistically significant (Fig. 6E-F). Taken together, these results suggest that SD can induce astrogliosis and microgliosis, which is consistent with the findings that SD increases proinflammatory cytokine levels in the brain.

Discussion

In the present study, we used an animal model of sleep deprivation that mimics chronically insufficient sleep as it often occurs in humans. We found that sleep deprivation leads to behavioral abnormalities, including anxiety and depressive-like behaviors. The consistent loss of sleep activates glial cells, which increase the release of pro-inflammatory cytokines in rats, leading to cognitive impairment and emotional disorders. Sleep deprivation also alters the transcript profile of circadian clock genes, which regulates physical, mental, and behavioral changes in several pathological processes. These observations indicate a new pathway through which sleep deprivation induces mental illness through the impairment the regulation of circadian clock genes and the induction of microglial proinflammatory cytokines and results in the progressive breakdown of the BBB to induce brain injury formation. These novel findings highlight the importance of circadian responses during the period of sleep loss and emphasize the contribution of circadian mechanisms for understanding mental disorders.

Accumulating evidence suggests that loss of sleep is closely related to neuropsychiatric disorders. The connection between sleep loss or deficits and mental disorders is not yet fully understood. SD impairs physiological and behavioral development by dysregulating proinflammatory cytokines, such as TNFα, IL-6 and IL-1β. Meanwhile, the serum and CSF levels of proinflammatory cytokines are also increased. However, the reason for the inflammatory cytokine upregulation in SD mice and its mechanism in SD-induced mental illness remain unknown. Astrocyte/microglial activation, along with the release of inflammatory cytokines, is considered, among other factors, to be the main source of neuroinflammation. In a recent study by Hurtado-Alvarado et al., it was reported that sleep deprivation for 10 consecutive days induces the overexpression of markers of reactive astroglia (GFAP) and microglia (Iba1) and disrupts the

blood-brain barrier via adenosine-mediated signaling(He et al., 2014a; Hurtado-Alvarado et al., 2016). Similarly, in our recent study of 3 days of acute sleep deprivation in rats, we observed reactive gliosis in astrocytic and microglial populations along with the secretion of proinflammatory mediators such as TNFα, IL-6 and IL-1β. Glial cell activation was evidenced by increased microglial reactivity and deviation from normal microglial morphology along with changes in the cell body, branching index and cell counting ratio of resting and activated microglial cells during SD. Interestingly, we found a significant increase in astrocyte immunoreactivity and the morphology of astrocytes was significantly altered, indicating astrocyte hypertrophy during SD-induced neuroinflammation. This is in agreement with earlier reports on the efficacy of chronic sleep loss against stress-induced gliosis.

Circadian clock genes are essential for driving and maintaining circadian rhythm. The suprachiasmatic nucleus (SCN) of the anterior hypothalamus is the internal clock that controls various physiological systems on a cycle of 24 hours. There are at least 9 core circadian clock genes (PER1, PER2, CRY1, CRY2, CLOCK, and BMAL1) that regulate central and peripheral circadian oscillators using transcriptional-translational feedback loops(Bass and Takahashi, 2010; Bellesi et al., 2017; Eckel-Mahan et al., 2013; Forde and Kalsi, 2017; Rosenwasser and Turek, 2015). Disruptions in circadian rhythm and alterations in circadian rhythm genes are associated with an increased risk of depression. A previous study also demonstrated the daily pattern of PER1, PER2, CRY1, CRY2, CLOCK and BMAL1 expression levels. In healthy people, circadian genes such as CLOCK and BMAL1 are expressed in a rhythmic fashion over the course of 24 hours. These genes cause hormonal changes that allow people to respond optimally to the demands of their day, especially the demands of wakefulness and sleep. In a study in mice, chronobiology researchers discovered that a mutation in the CLOCK gene leads to unusual behavior, including mania, hyperactivity and sleeplessness in mice(Siegel, 2005; Stickgold, 2005; Vyazovskiy and Faraguna, 2015; Vyazovskiy and Harris, 2013; Wadhwa et al., 2017; Yang et al., 2015; Zhu et al., 2012). This study suggests that mutations in the CLOCK gene may be part of the cause of mental illnesses. In fact, the sleep disturbances associated with depression often disrupt patients' mood and ability to function as much as the depression itself. However, the link between sleep disruption and depression is not well established. Our study demonstrated that SD-mediated depression is associated with reduced expression of circadian rhythm genes in the hypothalamus in an animal model of acute SD. We demonstrated that circadian rhythm genes contribute to the homeostatic aspect of sleep regulation. Indeed, changes in some circadian rhythm genes modify markers of sleep homeostasis, and an increase in homeostatic sleep drive alters circadian clock gene expression in the forebrain. Here, we established a possible mechanism by which SD can alter circadian rhythm clock genes expression by quantifying DNA binding of the core-clock transcription factors CLOCK and BMAL1 to the target circadian clock genes in animal model(Carreras et al., 2014; He et al., 2014b; Scammell et al., 2017; Takatoshi et al., 2010). Our research indicates that the disruption of the balance of circadian rhythm genes in the brain can induce mental illness caused by insufficient sleep. These data demonstrate that SD lead to abnormal circadian rhythm genes expression is regarded as an important contributing factor to the disruption of circadian rhythm, which is associated with numerous pathological processes in mental disease.

Conclusions

Taken together, the results of the present study show that consistent sleep loss is able to elicit anxiety and depressive-like behaviors in rats. SD induced neuroinflammation by dysregulating circadian clock genes and inducing microglial pro-inflammatory cytokines, and it resulted in the progressive breakdown of the BBB integrity to induce brain injury formation. With the high prevalence of anxiety and depression in society, animal models of sleep deprivation that result in anxiety and depressive-like phenotypes are critical for advancing our understanding of the mechanisms of these disorders and for finding effective treatments. This study suggests that correcting circadian rhythm genes regulation may be significant for mental disorders and that targeting these genes may provide an effective countermeasure against sleep loss induced neuroinflammation and mental illness.

Abbreviations

AQP-4: aquaporin-4; BBB: blood brain barrier; Bmal1: brain and muscle Arnt-like protein-1; Cry1: clock feedback gene cryptochrome 1; Cry2: clock feedback gene cryptochrome 2; Clock: circadian locomotor output cycles kaput; Dbp: D-box-binding protein; GFAP: astrocyte marker glial fibrillary acidic protein; Iba-1: Ionized calcium-binding adapter molecule 1; IL-1β: interleukin-1β; IL-6: interleukin-6; TNF-α: tumor necrosis factor α; Per1/Per2: period genes1/2; Rev-erbα: clock-targeted genes nuclear receptor; RORα: retinoic acid receptor-related orphan receptors α; TJ: tight junction; SCN: suprachiasmatic nucleus; SD sleep deprivation

Declarations

Acknowledgments

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Authors contribution

Xing Chen: Performed research, Analyzed data; Yanzhao Zhou: Performed research, Analyzed data; Xuan Xu: Contributed new reagents or analytic tools Mengnan Ding: Contributed new reagents or analytic tools; Yifan Zhang: Analyzed data; Meiru Hu: Contributed new reagents or analytic tools; Xin Huang: Designed research, Performed research, Analyzed data, Wrote the paper; Lun Song: Contributed new reagents or analytic tools, Analyzed data, Revised the paper.

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Ethics Approval and Consent to Participate

All procedures performed in studies involving animals were in accordance with the ethical standards of the institutional research committee. Rats were maintained at the animal facility with free access to water and food in accordance with institutional guidelines. The Institutional Animal Care and Use Committee (IACUC) of the Academy of Military Medical Sciences approved all experiments involving rat.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

Authors make readily reproducible materials described in this manuscript, including all relevant raw data, freely available to any scientist wishing to use them, without breaching participant confidentiality.

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Figures

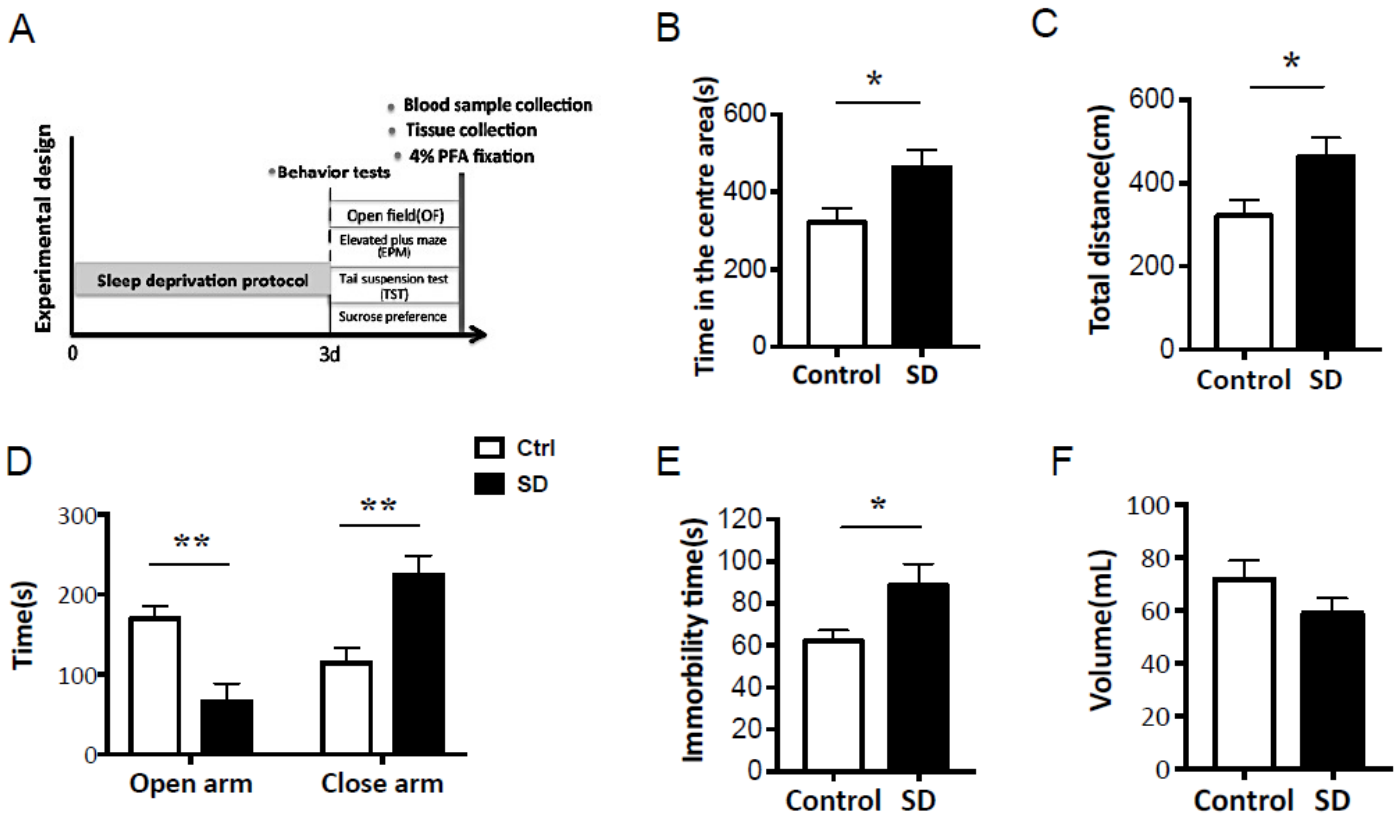

Figure 1

Sleep deprivation induced anxiety and depressive-like behaviors. (A) Schematic of the experimental design. (B-C) Sleep deprived animals spent less time in the center area in the OF test. (D-E) Sleep deprived animals spent more time in closed arm and reduced the entrance to open arms in EPM test. (F) SD significantly reduced the immorality time in TST test. (G) Sleep deprived animals shown the decreased sucrose consumption in SPT test. Values are presented as the mean ± S.E. (n=6); unpaired student t-test, *p < 0.05, **p < 0.01 versus control group.

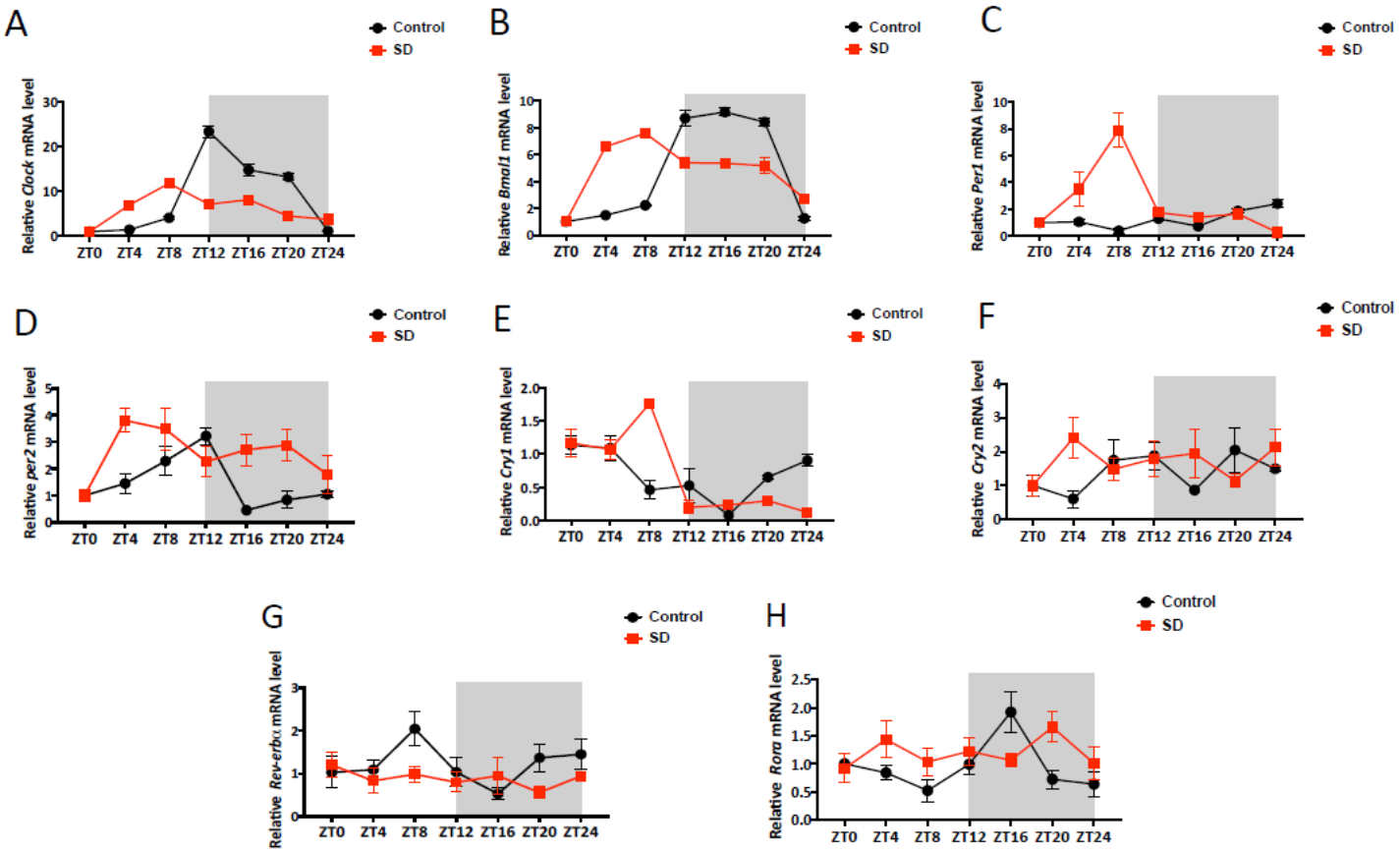

Figure 2

Transcriptional profiles of circadian clock genes from hypothalamus collected around the clock after SD. The total RNA from hypothalamus was prepared, and mRNA expression levels of clock genes were determined by quantitative real-time PCR. (A-H) The vertical axis shows normalized mean values ± S.E.M (n=3-4). The horizontal axis shows the sampling ZTs across 24h, after which the animals were killed and samples collected. The shaded area illustrates the dark phase of the day from ZT 12 to ZT 0. All conditions were plotted as relative change using ZT 0 as baseline value.

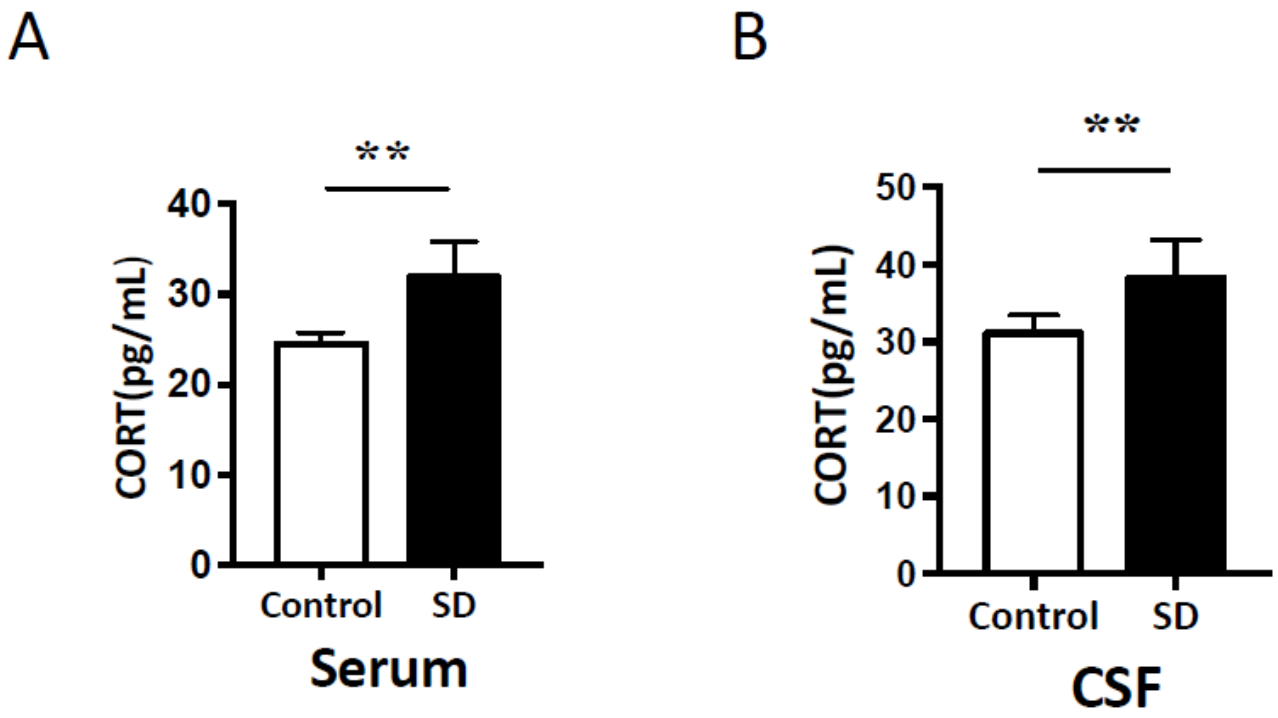

Figure 3

Sleep deprivation increased the level of cortisol in rats. (A-B) The level of cortisol in the serum and cerebrospinal fluid was measured by ELISA test. Values are presented as the mean ± S.E. (n = 6); unpaired student t-test, *p < 0.05, **p < 0.01 versus control group.

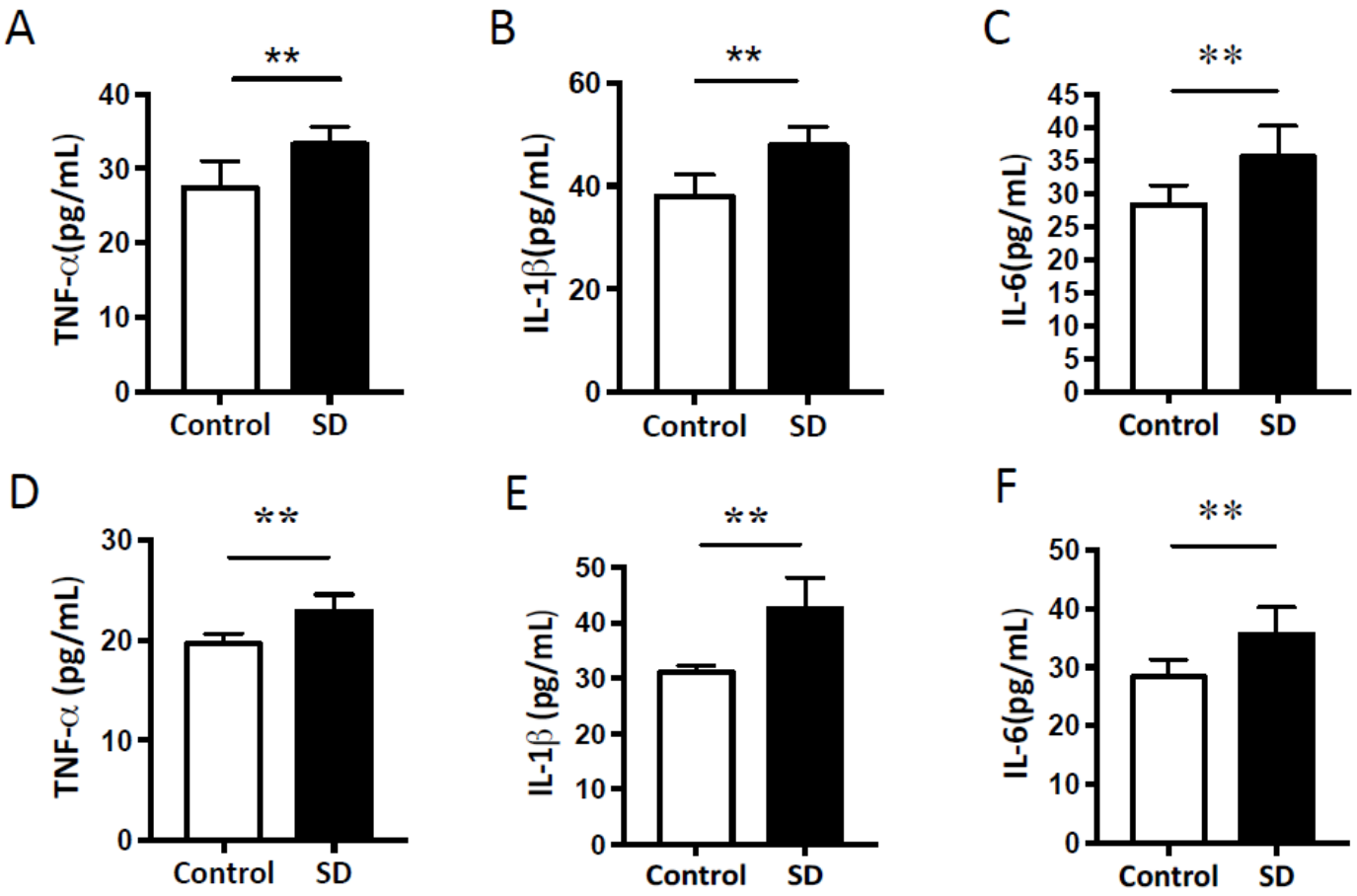

Figure 4

Sleep deprivation increased the levels of inflammatory cytokines in rats. (A-C) The level of TNF-α, IL-1β, and IL-6 in the serum after 3 days of SD. (D-F) The level of TNF-α, IL-1β, and IL-6 in the cerebrospinal fluid after 3 days of SD. Values are presented as the mean ± S.E. (n=5); unpaired student t-test, * $p<0.05$ compared with the control group, ** $p<0.01$ compared with the control group.

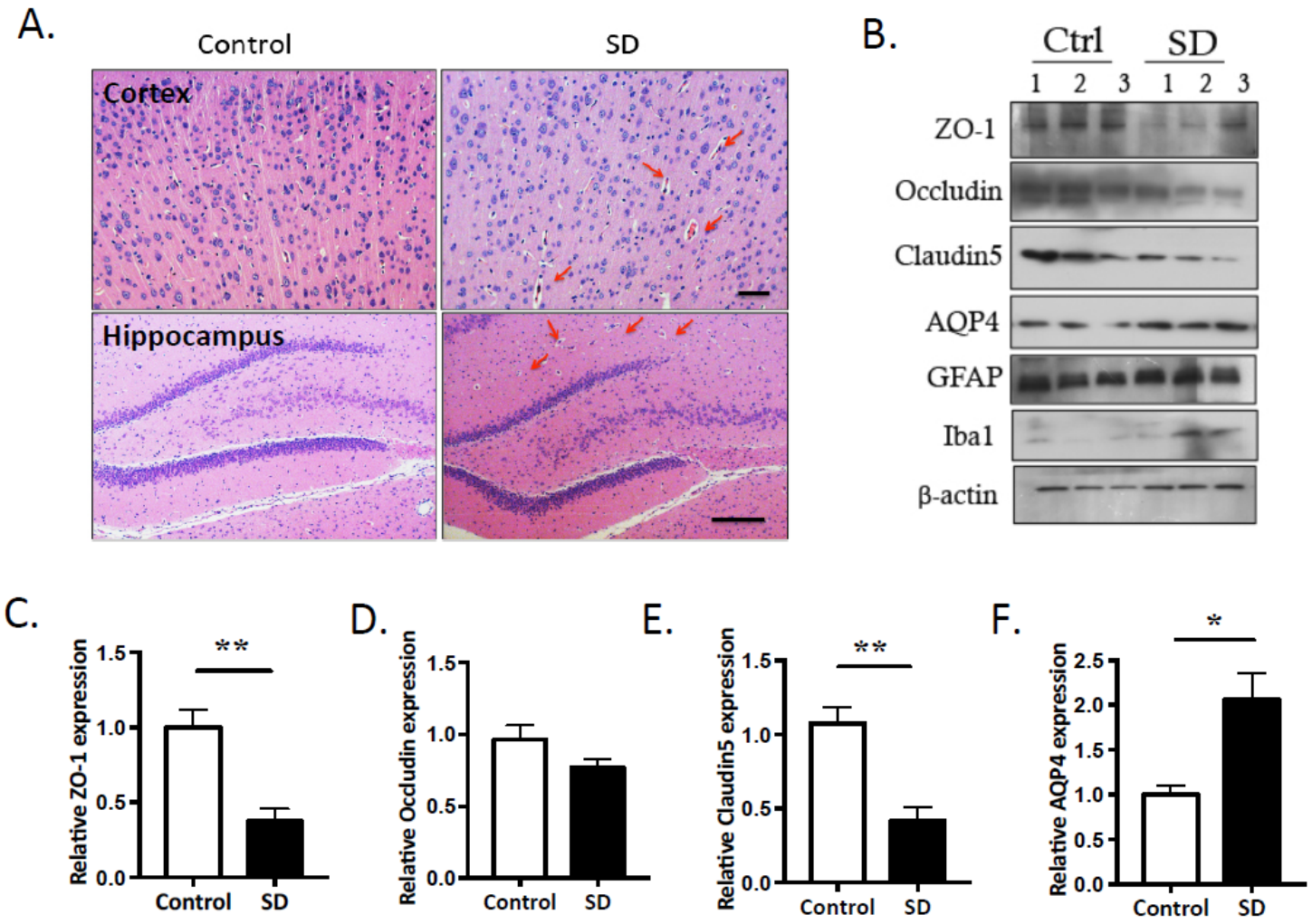

Figure 5

Sleep deprivation increased the levels of inflammatory cytokines in rats. (A-C) The level of TNF-α, IL-1β, and IL-6 in the serum after 3 days of SD. (D-F) The level of TNF-α, IL-1β, and IL-6 in the cerebrospinal fluid after 3 days of SD. Values are presented as the mean ± S.E. (n=5); unpaired student t-test, * $p<0.05$ compared with the control group, ** $p<0.01$ compared with the control group.

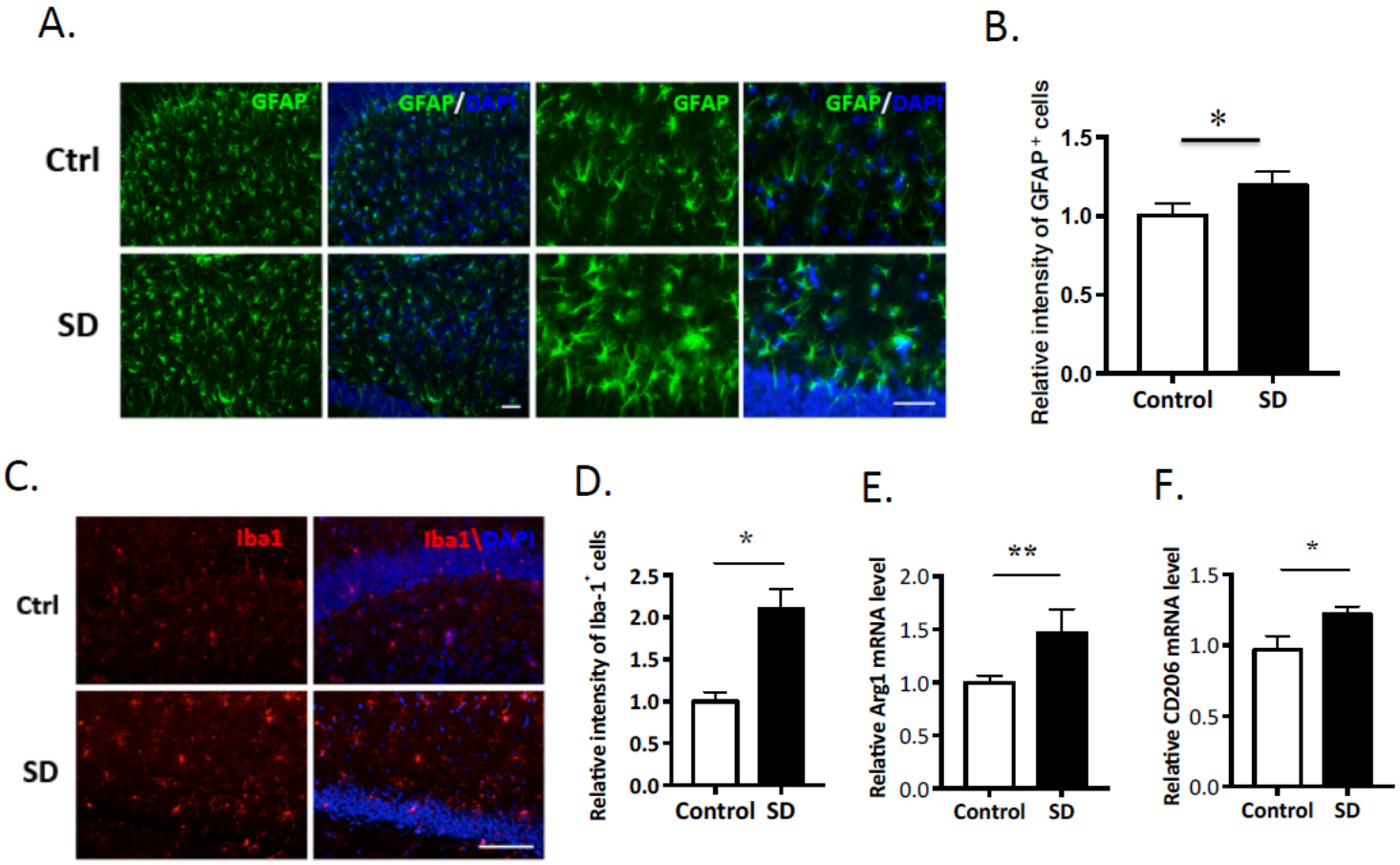

Figure 6

SD-induced inflammatory responses impair the blood-brain barrier in rats. (A) Representative image of HE staining in the brain. (B) Representative pictures of Western blot for ZO-1, CLAUDIN5, OCCLUDIN and AQP-4 expression in hypothalamus after 3 days of SD. (C-F) The quantitative analysis of Western blot for ZO-1, CLAUDIN5, OCCLUDIN and AQP-4 expression. Data are presented as the mean ± S.E. (n=6); * unpaired student t-test, $p<0.05$ compared with the control group, ** $p<0.01$ compared with the control group.