

Original Research

Quercetin Modulates Sleep Architecture and Homeostatic Recovery by Inhibiting Neuronal Hyperactivity and Microglial Activation in Mice

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Abstract

Background: Quercetin is a naturally occurring flavonoid widely distributed in plants that exhibits various biological activities, including anti-inflammatory, antioxidant, and neuroprotective effects. It exhibits a potential role in sleep regulation and homeostasis; however, its specific effects on sleep-wake cycles and underlying mechanisms remain unelucidated. **Methods:** To systematically investigate the regulatory role of quercetin in sleep architecture and homeostatic recovery, polysomnography (PSG) was used to monitor sleep parameters in mice under normal circadian rhythms and acute sleep deprivation (ASD). Immunofluorescence staining was performed to assess the expression of cellular proto-oncogene protein Fos (c-Fos) and microglial activation in sleep-related brain regions, including the medial prefrontal cortex (mPFC), nucleus accumbens (NAc), bed nucleus of the stria terminalis (BNST), paraventricular thalamic nucleus (PVT), hippocampal dentate gyrus (DG), basolateral amygdala (BLA), and periaqueductal gray (PAG). **Results:** Under normal circadian conditions, high-dose quercetin promoted non-rapid eye movement (NREM) sleep in mice. In ASD models, quercetin enhanced NREM sleep rebound during the early recovery phase. It sustained higher levels of wakefulness during the subsequent light phase, exhibiting its dual role in accelerating homeostatic recovery while balancing circadian arousal. Immunofluorescence analyses showed that quercetin markedly suppressed c-Fos expression in the mPFC, BLA, and PVT under sleep-deprived conditions. Additionally, it inhibited microglial activation in the mPFC and NAc. **Conclusion:** These results mechanistically associate the sleep-regulatory effects of quercetin with its dual inhibition of neuronal hyperactivity in sleep-associated brain regions and neuroinflammatory responses. Altogether, this study identifies quercetin as a novel natural modulator of sleep homeostasis, underscoring its therapeutic potential for sleep disorders via anti-excitatory and anti-inflammatory mechanisms.

Keywords: quercetin; sleep homeostasis; polysomnography; sleep deprivation; c-Fos; microglia

1. Introduction

Sleep is a fundamental physiological process essential for maintaining nervous system function and immune homeostasis, with sleep homeostasis regulation involving a bidirectional interplay with the neuroimmune network [1,2]. Clinical and animal studies suggest that disrupting sleep homeostasis can activate the immune system, exacerbate the increase in peripheral proinflammatory cytokines, and stimulate microglial activation within the central nervous system (CNS), thereby increasing the risk of infectious, autoimmune, and neurodegenerative diseases [3,4]. Microglia, the primary immune cells in the brain, regulate wake-sleep cycles by modulating neuronal activity in specific brain regions [5,6]. For instance, the activation of inhibitory G-protein signaling within microglia has been shown to promote sleep. Conversely, sleep states can regulate the morphology of microglia. Notably, animal studies have associated enhanced neuronal activity during wakefulness with elevated microglial branch extension and dy-

namic synaptic interactions, whereas microglia exhibit a more quiescent morphology during sleep. These findings closely associate microglial morphology with synaptic plasticity during the sleep-wake cycle [7–9]. Microglia undergo a morphological shift toward a proinflammatory phenotype, characterized by reduced branching and increased phagocytic activity, during sleep deprivation (SD). This shift, along with inflammatory infiltration in the meninges and brain parenchyma, reveals that sleep states influence immune responses by regulating microglial morphology [10]. Therefore, exploring approaches to maintain sleep homeostasis through immune system modulation provides a theoretical foundation for developing “anti-inflammatory–sleep-promoting” intervention strategies.

Quercetin, a natural flavonoid abundantly present in Chinese herbal medicines, is well known as its anti-inflammatory, antioxidant, and neuroprotective properties [11,12]. Recent study has shown that quercetin nanoparticles can modulate the gut-brain axis, attenuate the translo-



cation of proinflammatory cytokines into the brain, and improve sleep quality [13]. Additionally, quercetin has been reported to alleviate SD-induced behavioral alterations by inhibiting the inflammatory pathway in the hippocampus and restoring brain-derived neurotrophic factor-mediated neuroplasticity in SD models [14]. The anti-neuroinflammatory effects of quercetin mainly include suppression of the nuclear factor- κ B and mitogen-activated protein kinase signaling pathways, reduction in proinflammatory factor levels (including interleukin [IL]-1 β , tumor necrosis factor [TNF]- α , and nitric oxide [NO]), and up-regulation of anti-inflammatory cytokines such as IL-10 [15]. Furthermore, quercetin has been shown to alleviate SD-induced hippocampal neuronal pyroptosis by inhibiting the activation of the nucleotide-binding domain, leucine-rich repeat, and pyrin domain-containing protein 3 (NLRP3) inflammasome [16], thereby enhancing cognitive function through microglial regulation in the hippocampus [17]. Notably, quercetin regulates microglial activation to improve memory impairment, highlighting its significant neuroprotective role [18,19]. Hence, the inhibitory effect of quercetin on microglia may serve as a central mechanism linking immune regulation and sleep improvement [20].

This study aimed to employ polysomnography and SD paradigms to systematically investigate quercetin-mediated reshaping of sleep architecture in mice under both basal and homeostatic imbalance conditions. Additionally, the effects of quercetin on brain region-specific neuronal activity and microglial dynamics were investigated to elucidate the “natural compound–neuroimmune–sleep homeostasis” regulatory axis and provide mechanistic insights for interventions in sleep disorders.

2. Materials and Methods

2.1 Mice

Adult male C57BL/6J mice (age: 8–10 weeks) were obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd. and housed in a specific pathogen-free (SPF)-grade animal facility under a 12-h light-dark cycle (light on: 7:00–19:00) and controlled room temperature (24–26 °C), with ad libitum access to food and water. Mice were acclimated to the housing environment for 1 week before experimental manipulation to minimize stress. During acclimation and experimental periods, animals were monitored daily for general health status in accordance with the Guidelines for the Care and Use of Laboratory Animals (8th edition, National Institutes of Health, Bethesda, Maryland, USA). Animals were euthanized via intraperitoneal injection of an overdose of sodium pentobarbital (100 mg/kg body weight, Sigma-Aldrich, St. Louis, MO, USA; Cat. No.: P3761). All procedures involving animals were approved by the Henan Provincial Animal Care and Use Committee and conducted in accordance with the experimental guidelines of the Animal Experimentation Ethics

Committee of Xinxiang Medical University (No. XYLL-20240390), China.

2.2 EEG-EMG Electrodes Implantation

The experimental mice were anesthetized via intraperitoneal injection (i.p.) of ketamine (100 mg/kg; Cat. No. K-002; Supelco, Merck KGaA, St. Louis, MO, USA) and diazepam (5 mg/kg; Cat. No. D-907; Supelco, Merck KGaA), which was dissolved in 0.9% saline. The injection was administered at a volume of 0.1 mL/10 g of body weight. Subsequently, the implantation of electroencephalogram (EEG) and electromyography (EMG) electrodes was performed with a stereotaxic apparatus (Model No. 68803; RWD Life Science Co., Ltd., Shenzhen, China). The EEG recording electrode was placed in the medial prefrontal cortex (mPFC) at the coordinates: Bregma anteroposterior (AP) + 1.75 mm, mediolateral (ML) + 0.45 mm, with the EEG reference electrode implanted on the surface of the cerebellar skull. Two EMG electrodes were inserted into the bilateral neck muscles. The electrodes were secured with dental cement (Super-Bond C&B, Cat No.: FG2971, N Corporation, Moriyama, Shiga Prefecture, Japan). After surgery, the mice were returned to their home cages for recovery.

2.3 Polysomnographic Recording and Analysis

After EEG-EMG electrode implantation, the mice were allowed to recover for 1 week and then placed in a sound-attenuated chamber to adapt the resistance of the electrical commutator for 3 days. The polysomnographic recorder (Model No. Neurokey, Nanjing Greathink Medical Technology Co., Ltd., Nanjing, China) synchronously recorded EEG and EMG signals at a sampling frequency of 1000 Hz. The manual scoring of sleep states was performed following downsampling of the signals to 250 Hz. The sleep-wake states were classified into wakefulness, non-rapid eye movement (NREM) sleep, and rapid eye movement (REM) sleep based on the EEG and EMG signals. Wakefulness was recognized by high-frequency, low-amplitude EEG activity and high-amplitude EMG activity. NREM sleep was recognized by low-frequency (0.5–4 Hz), high-amplitude EEG dominated by delta waves and low-level EMG activity. REM sleep was recognized as low-amplitude EEG with theta wave dominance (4–9 Hz) and near-absent EMG activity.

Following manual scoring to identify NREM sleep states, EEG signals were subjected to fast Fourier transform (FFT) with a time window (4-second window length) to calculate the power spectral density (PSD, unit: $\mu\text{V}^2/\text{Hz}$). The delta wave band was defined as 0.5–4 Hz, and the gamma wave band as 30–80 Hz.

Sleep latency was defined as the duration from the initiation of EEG-EMG signal acquisition to the mouse's first transition into NREM sleep. If NREM sleep was not detected in the first hour, the latency was recorded as 3600

s. To minimize artifact interference, the first episode of NREM sleep was only included in the analysis if it was sustained continuously for ≥ 2 min; transient sleep events (< 2 min) were considered invalid fluctuations during the wakefulness period.

2.4 Sleep Deprivation Procedures

The mice were subjected to acute sleep deprivation (ASD) or chronic sleep deprivation (CSD) using the Rodent Sleep Deprivation System (Model XR-XS108; Shanghai XINRUAN Information Technology Co., Ltd., Shanghai, China). The mice were allowed to move freely with ad libitum access to food and water. Sleep deprivation was initiated at 7:00 during the light phase. The sleep deprivation rods rotated unidirectionally at 10 revolutions per minute (rpm). A 5-min interval was maintained between each full rotation of the rods. Each sleep deprivation period lasted 6 h.

2.5 Preparation and Administration of Quercetin

Quercetin (Cat. No. S2391; Selleckchem, Houston, TX, USA) was dissolved in freshly opened dimethyl sulfoxide (DMSO; Cat. No. D8371; Solarbio Science & Technology Co., Ltd., Beijing, China). Quercetin working solutions were then formulated at concentrations of 20 mg/kg and 200 mg/kg. The diluent for working solutions was prepared by sequentially mixing 5% DMSO, 40% polyethylene glycol 300 (PEG300; Solarbio Science & Technology Co., Ltd., Beijing, China; Cat. No.: IP9020), 5% Tween 80 (Solarbio Science & Technology Co., Ltd.; Cat. No.: IT9000), and 50% ddH₂O (DNase/RNase-Free Water; Solarbio Science & Technology Co., Ltd.; Cat. No.: R1600).

For ASD, quercetin (20 mg/kg or 200 mg/kg body weight) was administered via i.p. 30 min before the end of the deprivation period. For CSD, quercetin was administered intraperitoneally 30 min before the end of the SD on day 7. Immediately after the SD, a 24-h sleep recording was conducted.

At 30 min before the start of the night phase (active period), the experimental mice were injected via i.p. with quercetin. The control mice were administered an i.p. injection of the vehicle (the diluent for working solutions). The EEG-EMG signal was recorded continuously for 24 h, starting 30 min after quercetin injection.

2.6 Immunofluorescence Staining

To observe the effects of quercetin on the cellular proto-oncogene protein Fos (c-Fos) expression and microglial activation in the brain, quercetin was administered 30 min before the end of SD. After 60 min of quercetin administration, the mice were subjected to transcardiac perfusion. Subsequently, the brain tissues were harvested and subjected to post-fixation with 4% paraformaldehyde (PFA) for 8–12 h. Then, 40- μ m-thick coronal brain sections were prepared by using an automated vibratome

(Model: VT1200S; Leica Biosystems, Nussloch, Baden-Württemberg, Germany). The brain sections were collected into 24-well plates, and 4–6 slices containing the target brain regions were selected for immunofluorescence staining.

The brain sections were washed three times with 0.01 M phosphate-buffered saline (PBS), with each wash lasting 10 min. Then, the sections were blocked with 0.01 M PBS containing 0.3% Triton X-100 and 10% goat serum for 90 min at room temperature (RT). Following the removal of the blocking solution, the sections were incubated overnight at 4 °C with primary antibodies. The primary antibodies included: ionized calcium-binding adapter molecule 1 (Iba1) (Wako Pure Chemical Industries, Ltd., Osaka, Japan; Catalog No.: 019-19741; Dilution: 1:1500) and c-Fos antibody (Synaptic Systems GmbH, Göttingen, Germany; Catalog No.: 226008; Dilution: 1:5000).

The brain sections were subsequently rinsed thrice with 0.01 M PBS, with each rinse lasting 10 min. Fluorescent secondary antibodies (Goat anti-rabbit Alexa Fluor 594; catalog number: A-11012, Thermo Fisher Scientific, Waltham, MA, USA; Dilution: 1:800) diluted in 0.01 M PBS were added, and the brain sections were incubated for 2 h at RT. After three additional PBS washes (10 min each time), the brain sections were incubated with 4',6-diamidino-2-phenylindole (DAPI; Roche, Basel, Switzerland; Cat. No.: 10236276001, 5 μ g/mL) for 15 min at RT. Then, the stained sections were mounted in 50% glycerol. The immunofluorescence images were acquired using NIS-Elements Advanced Research software (NIS-Elements AR 5.42.06; Nikon Instruments Inc., Tokyo, Japan), a dedicated software for the Nikon Ti2-E confocal microscope (Model: Ti2-E; Nikon Instruments Inc., Tokyo, Japan) with 10 \times and 20 \times objectives, employing two laser wavelengths (i.e., 405 nm and 561 nm). Image processing and quantitative analysis were performed using ImageJ software version 1.53e (National Institutes of Health, Bethesda, MD, USA).

2.7 Statistical Analysis

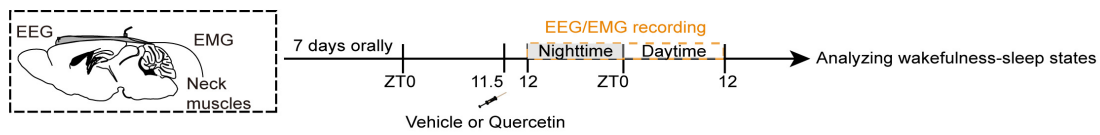
All statistical analyses were performed using GraphPad Prism 8.0 software (GraphPad Software, San Diego, CA, USA). The experimental data were presented as the mean \pm standard error of the mean (SEM). Statistical differences among the quercetin-treated groups were evaluated using one-way analysis of variance (ANOVA). Statistical significance between the groups was defined as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

3. Results

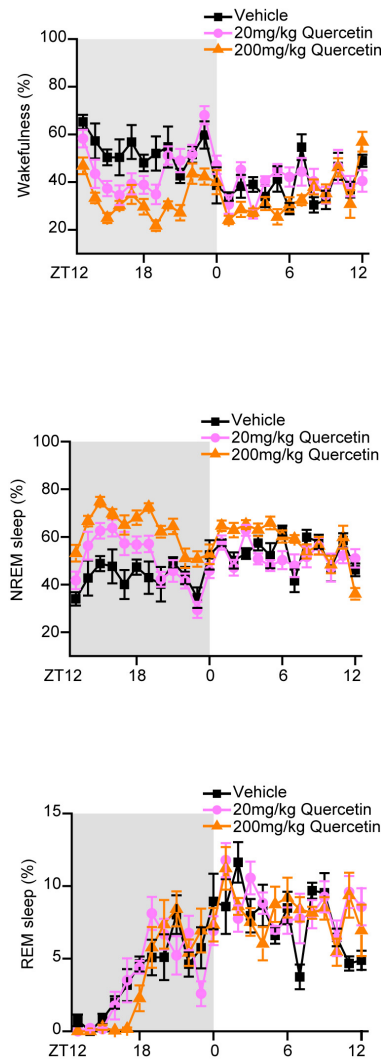
3.1 Quercetin Promotes Sleep in the Basal State

To investigate the regulatory effects of quercetin on sleep under physiological conditions, mice received a single i.p. injection of either low-dose (20 mg/kg) or high-dose (200 mg/kg) quercetin, and the control mice were administered a vehicle diluent. Following treatment, continuous

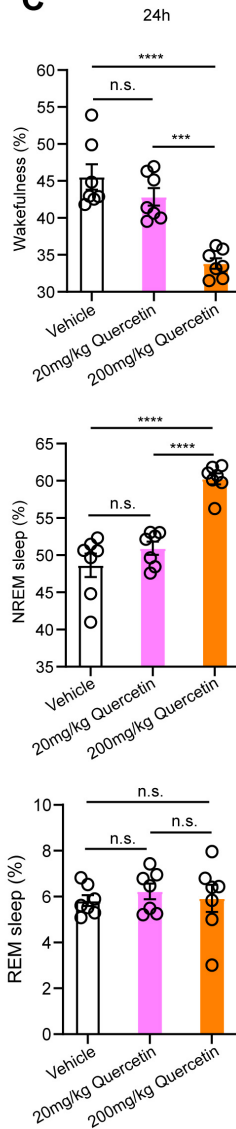
A



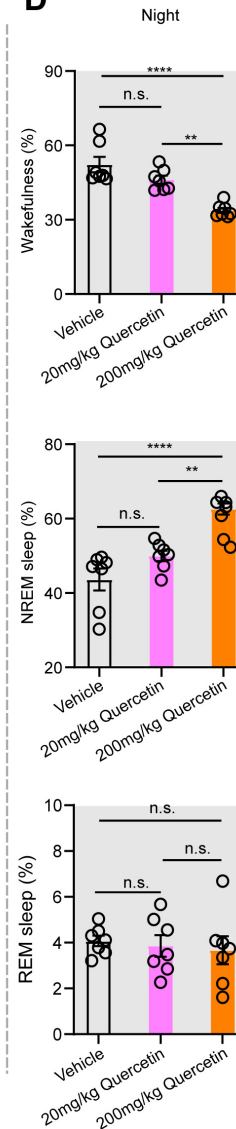
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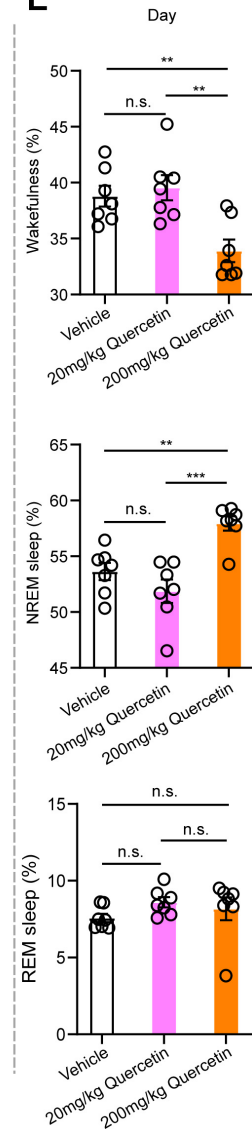


Fig. 1. Quercetin significantly increases the NREM sleep under basal conditions in mice. (A) Flow chart of electroencephalogram-electromyogram (EEG-EMG) recording after quercetin injection. (B) The line chart shows the percentages of wakefulness (upper), NREM sleep (middle), and REM sleep (lower) in mice after quercetin administration. (C–E) The statistical charts show the percentage of wakefulness (upper), NREM sleep (middle), and REM sleep (lower) over three time periods: 24 h (C), nighttime active phase (D), and daytime inactive phase (E). $n = 7$ per group. Each circle represents one mouse. The data are expressed as mean \pm standard error of the mean (SEM). The differences between groups were analyzed by one-way analysis of variance (ANOVA). ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, n.s., no significant difference. Vehicle, control group. 24 h, 24 hours. ZT, Zeitgeber Time; NREM, non-rapid eye movement; EEG, electroencephalogram; EMG, Electromyography; REM, rapid eye movement; SEM, standard error of the mean.

24-h EEG-EMG sleep signals were recorded (Fig. 1A). Notably, high-dose quercetin significantly increased the percentage of NREM sleep and decreased that of wakeful-

ness, with no significant differences observed in REM sleep (Fig. 1B,C). These effects were consistent across both active and inactive phases (Fig. 1D,E).

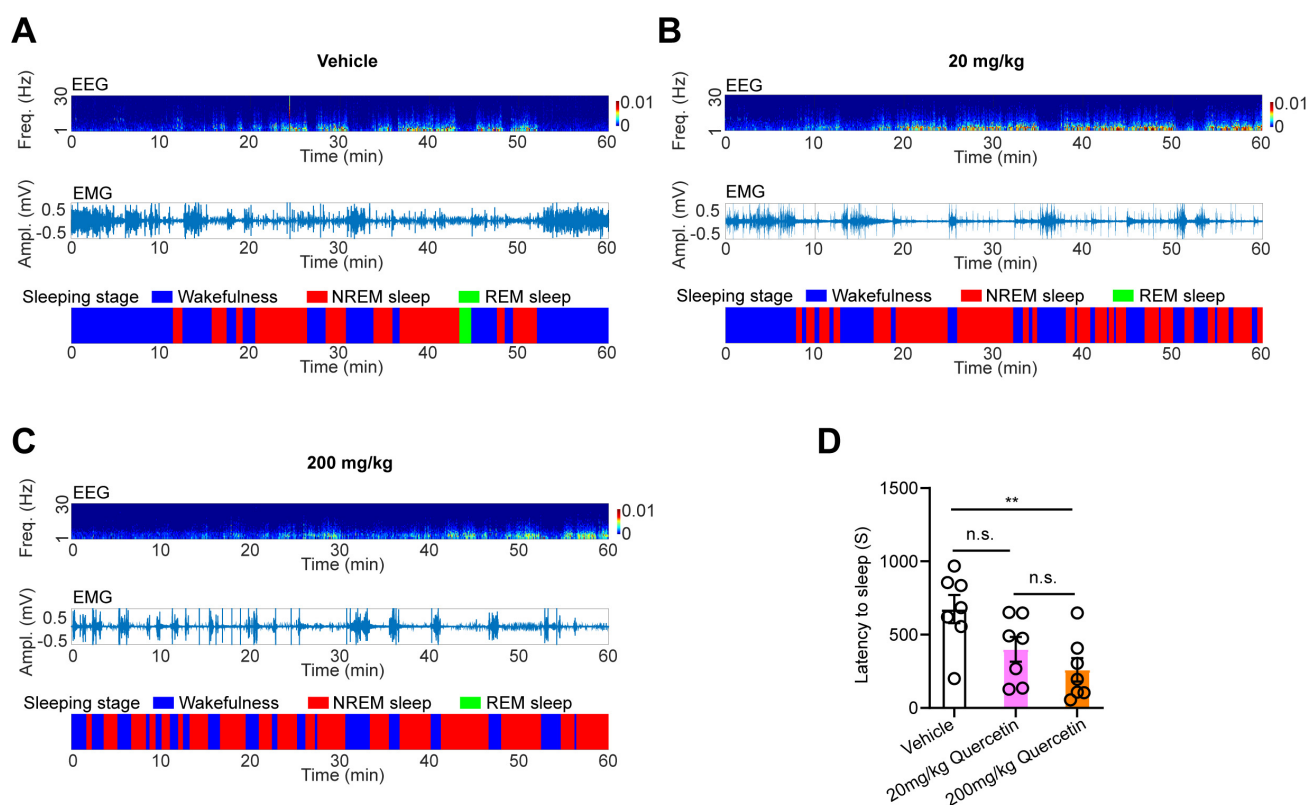


Fig. 2. Quercetin effectively reduces the sleep latency in mice. (A–C) Representative traces of EEG, EMG wave, and the sleeping stages in the vehicle control group (A), 20 mg/kg quercetin-treated group (B), and 200 mg/kg quercetin-treated group (C) during the first hour post-administration. (D) The statistical chart illustrates the sleep latency in vehicle-treated mice, 20 mg/kg quercetin-treated mice, and 200 mg/kg quercetin-treated mice. $n = 7$ per group. Each circle represents one mouse. The data are expressed as mean \pm SEM and analyzed by ANOVA. $**p < 0.01$, n.s., no significant difference. Ampl, Amplitude; Freq, Frequency.

To determine if quercetin facilitates the wakefulness-to-sleep transition in mice, post-treatment sleep latency during the 1st hour was analyzed (Fig. 2A–C). Sleep latency decreased dose-dependently, with high-dose quercetin significantly reducing sleep latency compared with the control group (Fig. 2D).

To further assess quercetin-mediated regulation of sleep pressure, EEG power spectra were evaluated during both day and night. Notably, the EEG power of delta (0.5–4 Hz) and gamma (30–80 Hz) waves did not differ significantly between quercetin-treated and control mice during both periods (Fig. 3A–F). Overall, these findings suggest that quercetin exerts an NREM sleep-promoting effect without elevating sleep pressure.

3.2 Quercetin Promotes Recovery of Sleep Homeostasis in ASD Mice

Sleep homeostasis is essential for overall physiological health, as disruption can impair immune function and increase susceptibility to infections and mood disorders. To determine whether quercetin regulates the maintenance of sleep homeostasis, we modeled a high-arousal state induced by sleep disruption using ASD in mice (Fig. 4A). Animals were subjected to a single-cycle of 6-h SD beginning at

zeitgeber time 6, followed by a 24-h EEG-EMG recording to evaluate sleep latency and sleep-wake states across the following four recovery phases: 1–6 h, 7–12 h, 13–18 h, and 19–24 h post-SD (Fig. 4B,C). Notably, quercetin did not affect sleep latency after ASD (Fig. 4B). During the first 1–6 h of recovery, the ASD group exhibited significantly increased NREM sleep and REM sleep compared with the vehicle group, concomitantly reducing wake time. In SD + quercetin mice, the percentage of NREM sleep was increased and REM sleep was decreased, while reducing wake time and increasing NREM sleep compared with that in the control group, with no difference in REM sleep (Fig. 4D).

During 7–12 h of recovery, the SD group exhibited increased NREM sleep and decreased wake time compared with the vehicle group. In quercetin + SD mice, no differences were observed in wakefulness, NREM, and REM sleep compared to the SD group. However, quercetin + SD mice spent less time in wakefulness and exhibited increased NREM and REM sleep compared with that in the control group (Fig. 4E). No statistical differences were observed among vehicle, SD, and quercetin groups during the 13–18 h recovery phase (Fig. 4F). Notably, at 19–24

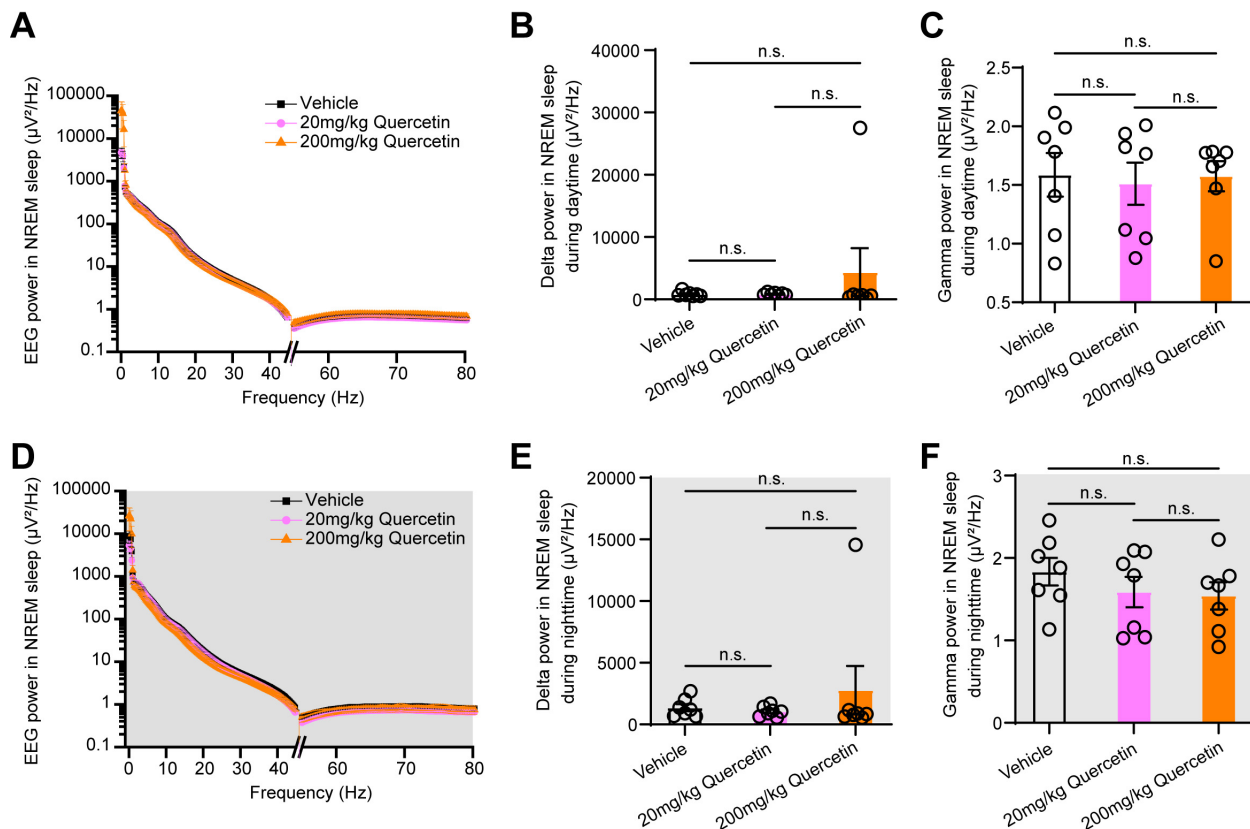


Fig. 3. Quercetin has no effect on the EEG power spectrum in mice under the basal state. (A,D) Statistical graph showing EEG power analysis of NREM sleep after quercetin injection during daytime (A) and nighttime (D). (B,C) Statistical charts show that quercetin had no significant effect on delta power (B) and gamma power (C) during daytime. (E,F) Statistical charts show that quercetin did not affect delta power (E) and gamma power (F) during nighttime. $n = 7$ per group. Each circle represents one mouse. Data are presented as mean \pm SEM and analyzed by ANOVA. n.s., no significant difference. Vehicle, control group.

h post-SD, the SD mice exhibited reduced wakefulness and increased NREM sleep compared to the control group, whereas quercetin + SD mice exhibited increased wakefulness and decreased NREM sleep compared with that in the SD mice, indicating a trend toward normalization (Fig. 4G).

Herein, EEG power spectra were analyzed during NREM sleep in 6-h intervals across the 24-h recovery period to assess the effects of quercetin on sleep pressure during recovery. Notably, delta (0.5–4 Hz) and gamma (30–80 Hz) wave powers among the control, SD, and quercetin + SD groups showed no significant differences across all recovery phases (Fig. 5A–L). These findings suggest that quercetin may have the potential to promote the recovery of sleep homeostasis following ASD without altering sleep pressure.

3.3 Quercetin Minimally Affects Post-CSD Sleep Homeostasis Recovery

The role of quercetin in restoring sleep homeostasis under CSD was examined in mice undergoing CSD for seven consecutive days, followed by 24-h sleep recording (Fig. 6A). No statistical differences were present in sleep latency among the control, CSD, and CSD + quercetin groups

(Fig. 6B). We then analyzed the 24-h recovery period to examine the proportion of wakefulness, NREM sleep, and REM sleep (Fig. 6C).

During the first 1–6 h of sleep recovery, the CSD group exhibited decreased wakefulness and increased NREM sleep compared to control mice. Unlike the ASD group, the CSD + quercetin group exhibited decreased REM sleep compared to the CSD group, with no significant differences in wakefulness and NREM sleep. Compared with the control mice, the CSD + quercetin mice experienced increased NREM sleep, following decrease in time spend in wakefulness and REM sleep (Fig. 6D). No significant differences were observed in wakefulness, NREM, and REM sleep among the control, CSD, and CSD + quercetin groups during the subsequent recovery phases (7–12, 13–18, and 19–24 h, Fig. 6E–G).

Additionally, post-CSD EEG power analysis revealed no significant differences in delta or gamma wave bands during NREM sleep (Fig. 7A–L). Altogether, these results suggest that a single administration of quercetin exerts minimal influence on restoring sleep homeostasis following CSD.

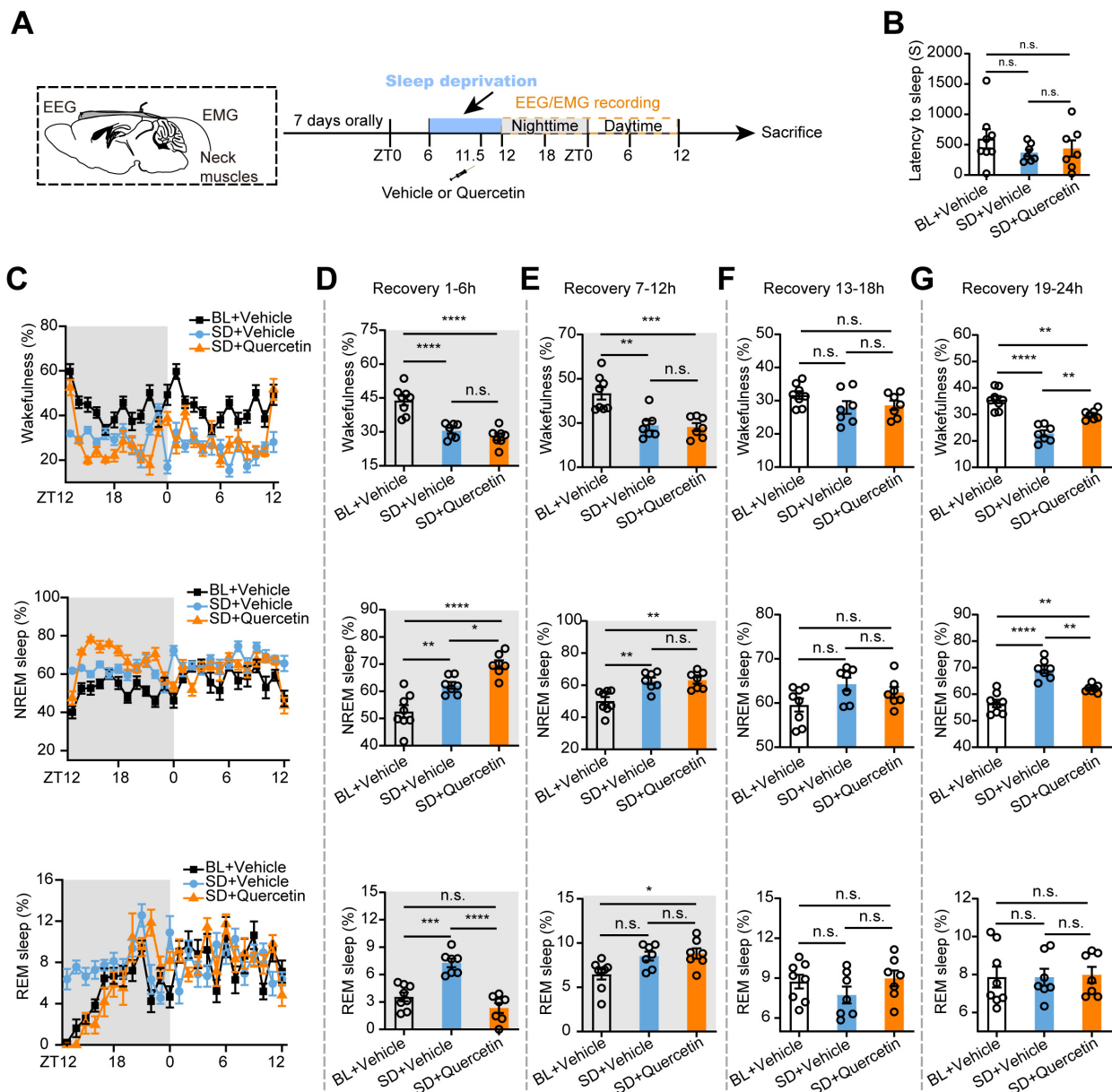


Fig. 4. Quercetin significantly improves sleep homeostasis in ASD mice. (A) Flow chart showing sleep recording after ASD. (B) The statistical chart shows that i.p. injection of quercetin has no significant difference in sleep latency after ASD. (C) The line chart shows the percentage of time spent in wakefulness (upper), NREM sleep (middle), and REM sleep (lower) during a 24-hour sleep recovery period in mice. (D–G) Statistical graphs show the percentage of wakefulness (upper), NREM sleep (middle), and REM sleep (lower) during the 1–6 h (D), 7–12 h (E), 13–18 h (F), and 19–24 h (G) recovery periods, respectively. BL + Vehicle: $n = 8$, SD + Vehicle: $n = 7$, SD + Quercetin: $n = 7$. Each circle represents a mouse. Data are expressed as mean \pm SEM and analyzed by ANOVA. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$, n.s., no significant difference. Vehicle, control group. ASD, acute sleep deprivation; i.p., intraperitoneal injection; BL, basal state; SD, sleep deprivation.

3.4 Quercetin Reduces SD-Induced Microglial Activation and c-Fos Expression

Based on the sleep homeostasis-restoring effect of quercetin after ASD, its impacts on neuronal activity and microglia activation in specific brain regions were explored. Mice received a single i.p. injection of quercetin (200 mg/kg) immediately after ASD, followed by immunofluorescence staining for c-Fos (Fig. 8A) and Iba1

(Fig. 9A). Examination included various key sleep-related brain regions, including the mPFC, nucleus accumbens (NAc), bed nucleus of the stria terminalis (BNST), paraventricular thalamic nucleus (PVT), hippocampal dentate gyrus, basolateral amygdala (BLA), and periaqueductal gray (PAG).

Notably, c-Fos staining revealed that quercetin attenuated the SD-induced c-Fos upregulation in the mPFC,

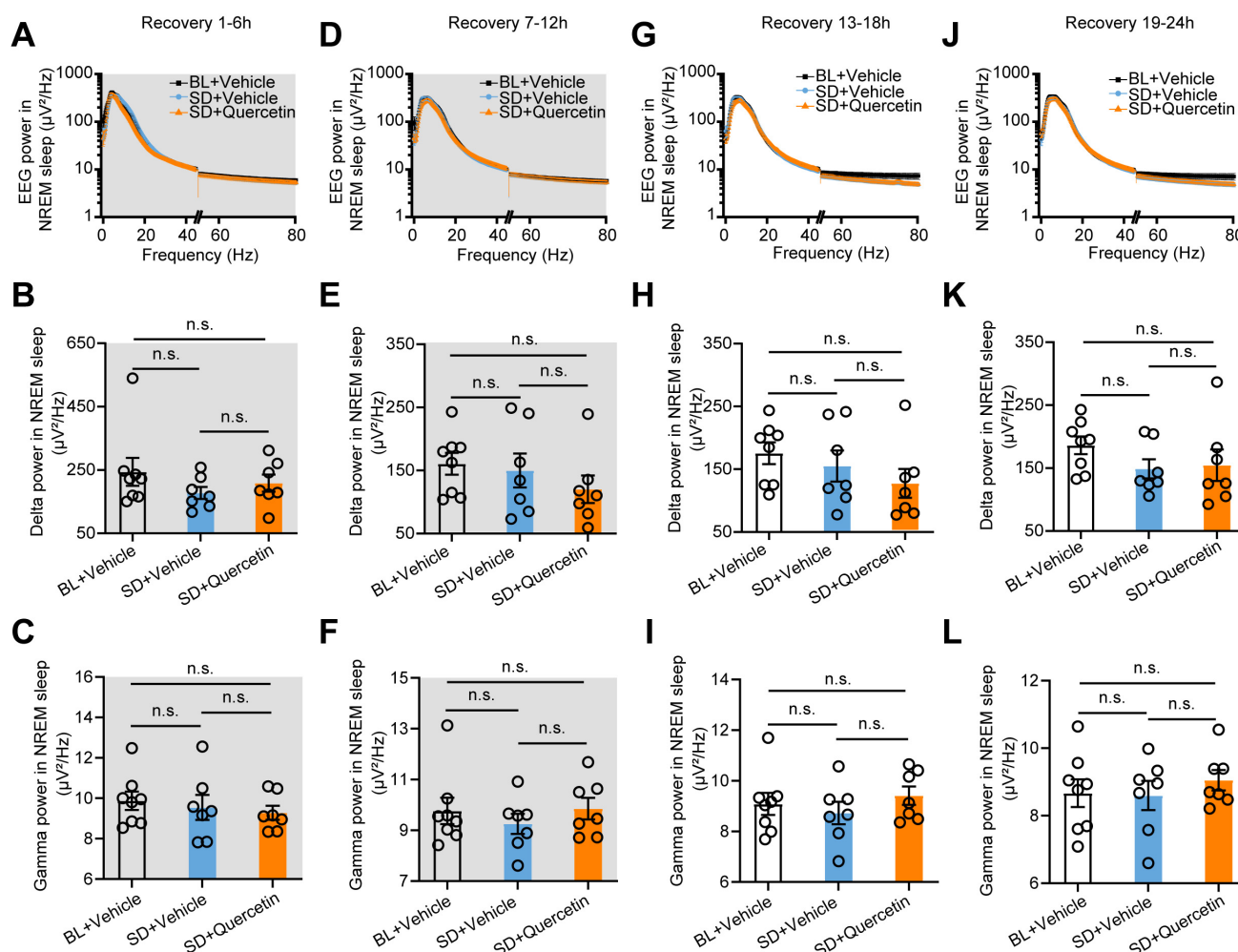


Fig. 5. Quercetin did not affect the EEG power spectrum during the sleep recovery period after ASD. (A,D,G,J) Statistical graphs show EEG power spectrum analysis of NREM sleep during the 1–6 h (A), 7–12 h (D), 13–18 h (G), and 19–24 h (J) of sleep recovery after acute sleep deprivation. (B,E,H,K) Statistical charts illustrate the effects of quercetin on delta power during the recovery periods of 1–6 h (B), 7–12 h (E), 13–18 h (H), and 19–24 h (K) after ASD. (C,F,I,L) Statistical charts show the effects of quercetin on gamma power during the recovery periods of 1–6 h (C), 7–12 h (F), 13–18 h (I), and 19–24 h (L) after ASD. BL + Vehicle: $n = 8$, SD + Vehicle: $n = 7$, SD + Quercetin: $n = 7$. Each circle represents a mouse. Data are presented as mean \pm SEM and analyzed by ANOVA. n.s., no significant difference.

PVT, and BLA, whereas it enhanced c-Fos expression in the BNST (Fig. 8B–I). Iba1 immunostaining revealed that quercetin significantly reduced SD-induced microglial activation, as indicated by decreased Iba1 immunoreactivity in the mPFC, NAc, and PAG (Fig. 9B–I). Overall, these results suggest that quercetin suppresses microglial activation and c-Fos expression in critical sleep-regulatory brain regions, potentially underpinning its modulatory effects on sleep homeostasis.

4. Discussion

This study aimed to systematically elucidate the regulatory effects of quercetin in sleep homeostasis and its underlying neuroimmunological mechanisms. Notably, quercetin exerts an NREM sleep-promoting effect under

basal conditions, and after ASD, it modulated sleep architecture, thereby suppressing excessive neuronal activation in specific brain regions and alleviating microglial inflammatory responses. Altogether, these findings provide novel insights into the mechanisms underlying the natural compound-mediated regulation of sleep and neuroimmunity.

Due to its limited blood–brain barrier (BBB)-penetrating ability, quercetin requires higher doses in studies on CNS mechanisms in mice compared to those in peripheral anti-inflammatory/antioxidant experiments. According to reports, doses of 10–200 mg/kg are considered effective for quercetin-mediated regulation of sleep–wake cycles in rodents [21,22]. Recent study on regulating CNS-mediated sleep regulation has consistently used a dose of

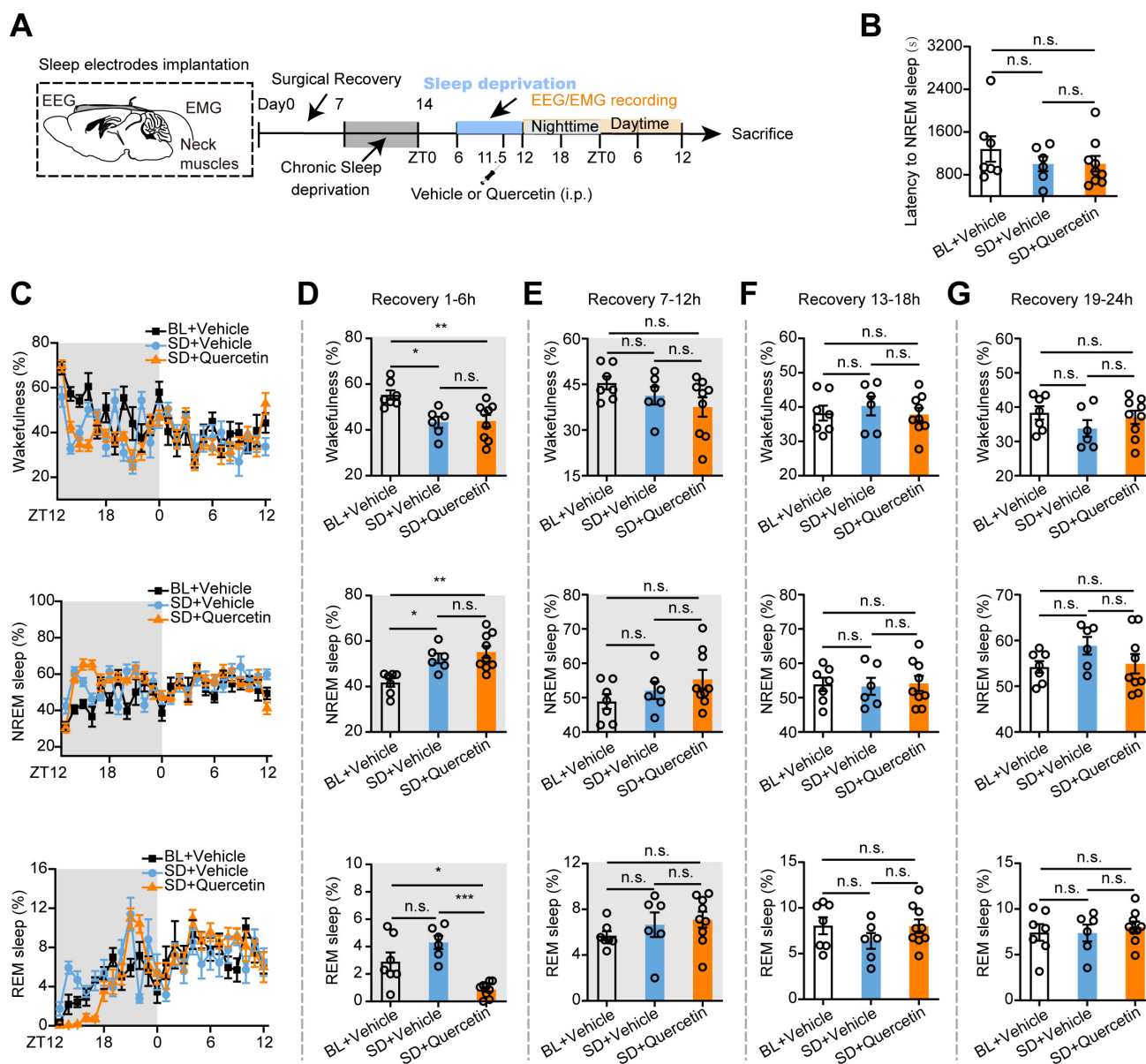


Fig. 6. Quercetin has few significant effects on sleep homeostasis in CSD mice. (A) Flow chart of sleep recording after CSD. (B) Statistical chart showing the sleep latency in CSD mice after quercetin treatment. (C) Percentages of wakefulness (upper), NREM sleep (middle), and REM sleep (lower) in different groups. (D–G) Statistical charts show the percentage of wakefulness (upper), NREM sleep (middle), and REM sleep (lower) during the 1–6 h (D), 7–12 h (E), 13–18 h (F), and 19–24 h (G) recovery periods, respectively. BL + Vehicle: n = 7, SD + Vehicle: n = 6, SD + Quercetin: n = 9. Each circle represents a mouse. Data are presented as mean ± SEM and analyzed by ANOVA. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, n.s., no significant difference. CSD, chronic sleep deprivation.

200 mg/kg, which effectively targets brain neurotransmitter systems to modulate sleep [23]. Regarding safety, a toxicity study on the hydroethanolic extract of *Dolichandra unguis-cati* L. leaves showed that a single, high-dose oral administration did not cause mortality or abnormal behaviors in male and female rats over 14 d. Furthermore, the rats exhibited normal weight gain, with no pathological abnormalities in core indicators of liver and kidney function [24]. Based on these findings, the 200 mg/kg dose setting (upper limit) was included in this study, which also exhibits basic safety.

A Study investigating quercetin's role in treating sleep deprivation-related memory impairment has shown that acute REM SD can activate inflammatory signaling pathways in the hippocampus [18]. A study by Wu *et al.* [13] reported that orally administered quercetin nanoparticles could regulate sleep duration under high-altitude conditions, providing critical evidence for developing drug treatments for altitude-related sleep disorders. The present study induced SD by disrupting sleep via the rotarod method and employed the i.p. method to administer quercetin,

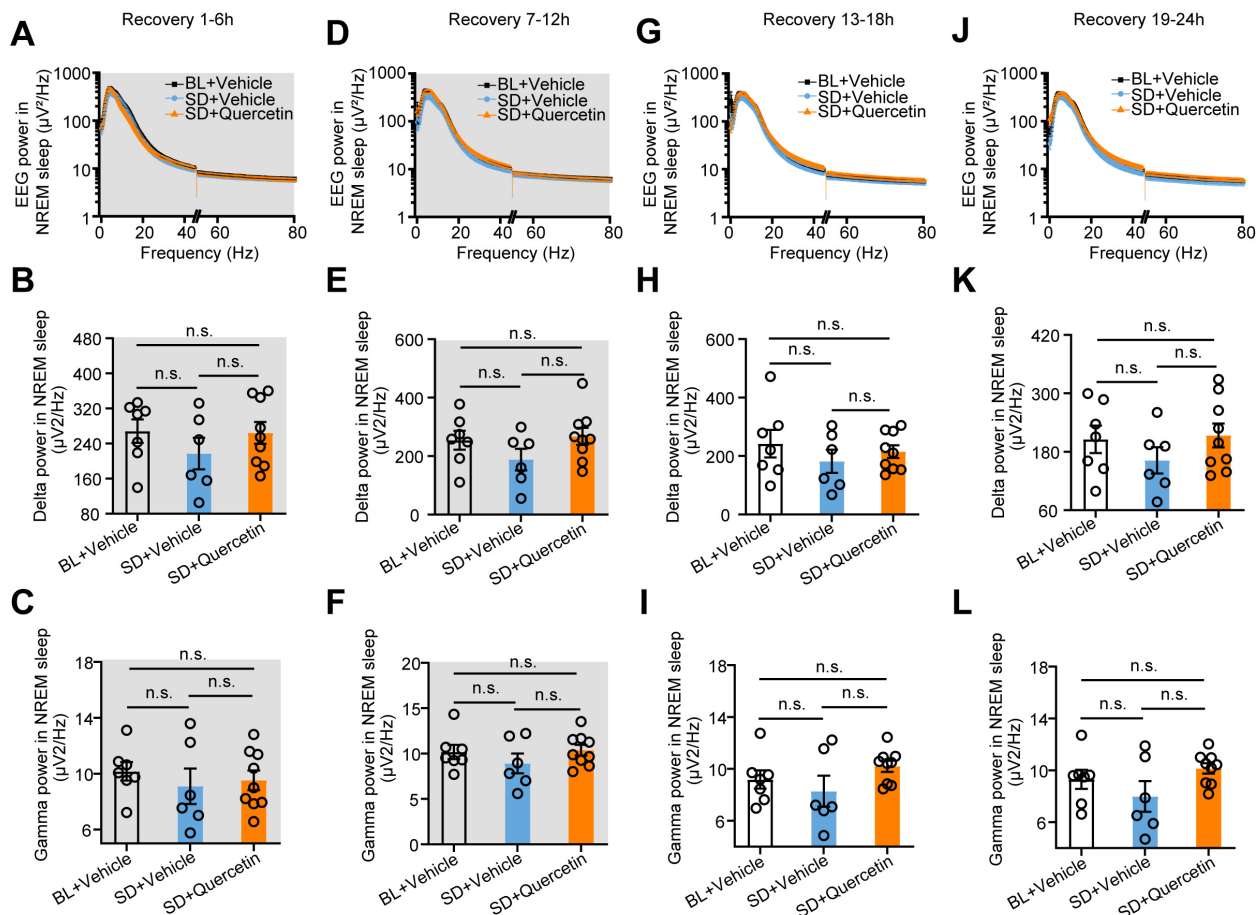


Fig. 7. Quercetin did not affect the EEG power spectrum in chronically sleep-deprived mice. (A,D,G,J) Statistical graphs showing EEG power spectrum analysis in NREM sleep during the 1–6 h (A), 7–12 h (D), 13–18 h (G), and 19–24 h (J) recovery periods after CSD. (B,E,H,K) Statistical charts show the effects of quercetin on delta power during the 1–6 h (B), 7–12 h (E), 13–18 h (H), and 19–24 h (K) sleep recovery periods after CSD. (C,F,I,L) Statistical charts show the effects of quercetin on gamma power during the sleep recovery periods of 1–6 h (C), 7–12 h (F), 13–18 h (I), and 19–24 h (L) after CSD. BL + Vehicle: $n = 7$; SD + Vehicle: $n = 6$; SD + Quercetin: $n = 9$. Each circle represents a mouse. Data are presented as mean \pm SEM and analyzed by ANOVA. n.s., no significant difference.

thereby expanding its application in modulating normal sleep rhythms and addressing both ASD and CSD. Additionally, this study investigated cellular activity and microglial activation in key brain regions involved in sleep regulation.

Despite accumulating evidence indicating the therapeutic potential of quercetin in sleep research, various challenges persist in its clinical translation. For instance, free quercetin can be enzymatically hydrolyzed in the gastrointestinal tract or be challenging to absorb owing to low solubility [25]. Nevertheless, recent advancements in nanoformulation technology have provided promising solutions to these issues. For example, quercetin-maize peptide nanoliposomes, synthesized using multifrequency pulsed ultrasonic methods, exhibited significantly enhanced quercetin-encapsulation efficiency, gastrointestinal stability, and transmembrane transport efficiency (they could penetrate the tight junctions of small intestinal epithelial cells) [25], thereby enhancing their potential for

CNS targeting. Regarding the clinical translation of neuropsychiatric disease treatment, quercetin-loaded chitosan nanoparticles (Qu-Ch NPs) have demonstrated notable advantages, including a low dose and high safety [26]. Low-dose Qu-Ch NPs exert strong neuroprotective effects by modulating antioxidant and anti-inflammatory pathways.

Advances in nose-to-brain delivery technology have further established an effective route for quercetin entry into the CNS. Notably, traditional oral and intravenous methods achieved extremely low quercetin concentrations in the brain due to the restrictive BBB. In contrast, intranasally delivered quercetin nanoparticles exhibited direct entry into the brain via the olfactory nerve pathway [27]. Notably, mitochondria-targeted quercetin nanoformulations have been shown to combat Alzheimer's disease-related ferroptosis through a triple mechanism of "iron chelation-pathway regulation-lipid protection" [28], offering strong experimental evidence for their potential in treating neurodegenerative diseases. However, regard-

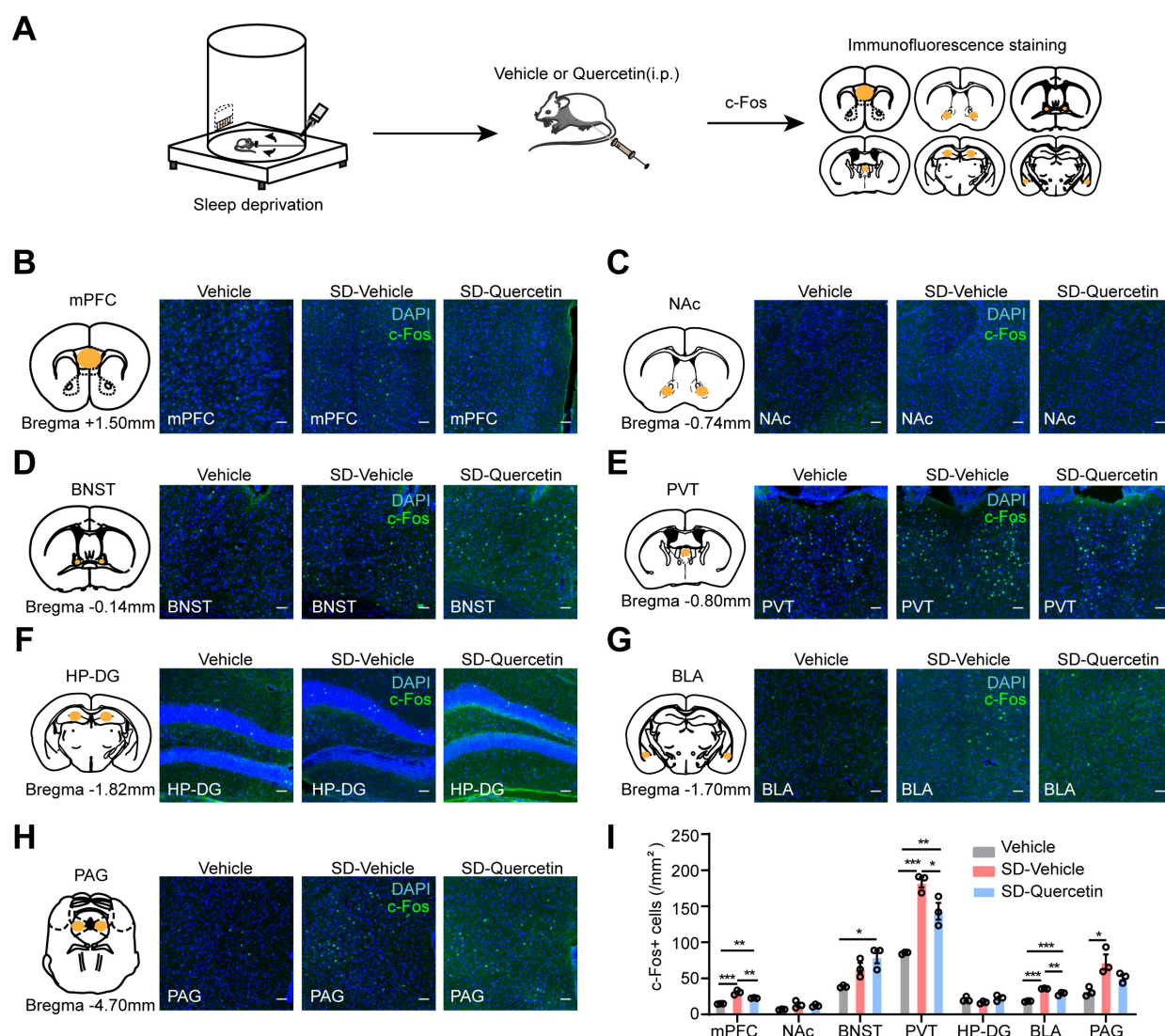


Fig. 8. Effects of quercetin on c-Fos expression in brain regions of acutely sleep-deprived mice. (A) Flow chart showing the effects of quercetin on c-Fos expression in brain tissues of acutely sleep-deprived mice. (B–H) Representative images show the expression of c-Fos in the mPFC (B), NAc (C), BNST (D), PVT (E), HP-DG (F), BLA (G), and PAG (H) of mice in the Vehicle, SD-Vehicle, and SD-Quercetin groups. Scale bar, 50 μ m. (I) Histograms display the number of c-Fos-positive cells in the mPFC, NAc, BNST, PVT, HP-DG, BLA, and PAG regions across the three groups. $n = 3$ per group. Each circle represents a mouse. Data are presented as mean \pm SEM and analyzed by ANOVA. * p < 0.05, ** p < 0.01, *** p < 0.001. c-Fos, cellular proto-oncogene protein Fos; mPFC, medial prefrontal cortex; NAc, nucleus accumbens; BNST, bed nucleus of the stria terminalis; PVT, paraventricular thalamic nucleus; HP-DG, hippocampal dentate gyrus; BLA, basolateral amygdala; PAG, periaqueductal gray. Fig. 8A created with Adobe Illustrator CS6 (Version 16.0.0; Adobe Inc., San Jose, California, USA).

less of their potential for clinical translation, further research is necessary on quercetin nanoformulations to clarify the correlation between “dose-central concentration-pharmacodynamic effect” and comprehensive long-term safety. Overall, optimizing quercetin nanoformulations and delivery systems can effectively overcome the major limitation of low bioavailability, highlighting their substantial potential for clinical application in treating neuropsychiatric diseases.

The power of delta waves (0.5–4 Hz) during NREM sleep is a classical indicator of sleep pressure dynamics. Additionally, sleep latency directly reflects the intensity of sleep drive and represents an intuitive metric of sleep pressure. Reportedly, the interaction between “homeostatic pressure (Process S)” and “circadian rhythm (Process C)” governs the sleep-wake balance [29]. Notably, Process S, also referred to as the “traditional homeostatic pathway” of sleep, has the following characteristic core feature: the longer the duration of wakefulness, the greater

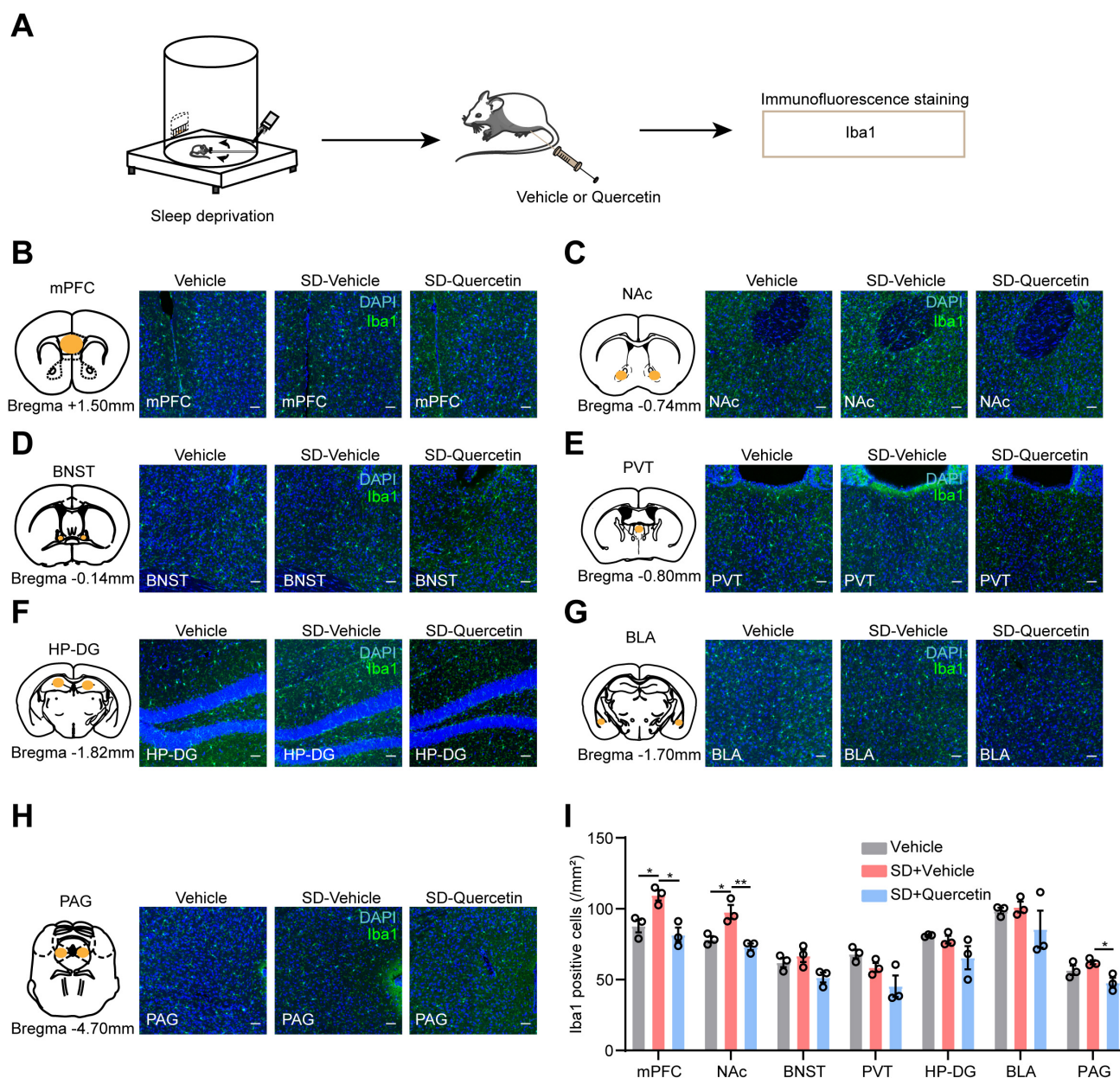


Fig. 9. Quercetin regulates the activation of microglia in brain regions of acutely sleep-deprived mice. (A) Experimental flow chart for evaluating the effect of quercetin on microglial activation after ASD. (B–H) Representative images showing the expression of Iba1 in the mPFC (B), NAc (C), BNST (D), PVT (E), HP-DG (F), BLA (G), and PAG (H). Scale bar, 50 μ m. (I) Histograms showing the number of Iba1-positive cells in the brain regions corresponding to (B–H). $n = 3$ per group. Each circle represents an individual mouse. Data are presented as mean \pm SEM and analyzed by ANOVA. * $p < 0.05$, ** $p < 0.01$. Iba1, ionized calcium-binding adapter molecule 1. Fig. 9A created with Adobe Illustrator CS6 (Version 16.0.0; Adobe Inc).

the accumulation of sleep pressure. Accumulated sleep pressure results in a compensatory increase in delta wave power during sleep, ultimately facilitating pressure dissipation [30,31]. During wakefulness, neuronal activity-associated metabolite accumulation, coupled with elevated proinflammatory cytokine levels, enhances neuronal excitability and synergistically promotes the buildup of sleep pressure [32]. Together, they act on specific brain regions, including the basal forebrain and hypothalamus, to inhibit

wake-promoting pathways, activate sleep-promoting pathways, and induce sleep [33]. Following sleep onset, slow-wave sleep (SWS) and delta wave activity direct the traditional homeostatic pathway into a pressure-dissipation phase, as this pathway activation relies on various factors beyond delta wave power alone, including adenosine concentration, sleep latency, and SWS proportion. It is important to emphasize that the traditional sleep homeostatic pathway does not regulate sleep independently.

Reportedly, sleep initiation involves two parallel pathways: the “homeostasis-dependent” and the “homeostasis-independent” pathways [34]. The traditional pathway relies on the accumulation of homeostatic factors, such as adenosine. In contrast, the homeostasis-independent pathway initiates sleep by directly regulating sleep-wake switching neurons, for example, through gamma-aminobutyric acid (GABA), which mediates receptor-driven inhibition of wake-promoting pathways [35]. Quercetin has been shown to act on the CNS primarily by inhibiting neuroinflammation and regulating oxidative stress levels [21,36].

In the present study, quercetin treatment reduced sleep latency under basal conditions; however, it exhibited no significant effect on post-SD sleep latency. Additionally, quercetin did not significantly affect delta wave activity during sleep under both baseline conditions and post-SD recovery phase. Integrating the aforementioned literature findings with the present results, we hypothesize that quercetin regulates sleep by modulating cerebral neuronal excitability and microglial activity, rather than interfering with sleep pressure accumulation reflected by delta waves. This finding is consistent with a previously reported study [21]. Additionally, this process is likely associated with the cortical-limbic system and brainstem multilevel neural circuits that maintain sleep-wake homeostasis. To clarify this association, the roles of key sleep-regulatory brain regions were delineated in this study.

Notably, sleep-wake homeostasis regulation depends on coordination among multilevel neural circuits spanning the cortex, limbic system, and brainstem. Insomnia is often associated with hyperactivity or disrupted network connectivity in different important brain regions. For instance, the mPFC has been shown to drive wakefulness by enhancing thalamocortical synchronization [37]. Similarly, the NAc regulates sleep-wake cycles primarily via dopaminergic and glutamatergic signaling, with hyperactivity of dopaminergic neurons potentially prolonging wakefulness in chronic stress or insomnia conditions [38]. Furthermore, the BNST and BLA integrate limbic inputs to modulate the sleep-wake balance. The BNST affects this balance by regulating GABAergic signaling. For example, pharmacological or optogenetic inhibition of GABAergic neurons in the BNST reportedly improved sleep continuity and reduced stress-induced wakefulness [39]. The BLA processes stress and emotional memory, structural and functional alterations and chronic insomnia [40,41]. Notably, acute stress can trigger the PVT, a relay station connecting the cortex, limbic system, and brainstem, to activate glutamatergic projections to the central amygdala, thereby promoting hyperarousal [42]. Additionally, the hippocampus and ventrolateral PAG (vlPAG) further contribute to sleep-wake regulation via homeostatic and neuroprotective mechanisms. For instance, the hippocampus can suppress excessive stress responses via the hypothalamic-pituitary-adrenal (HPA) axis, and its dysfunction has been associated with

anxiety and depression comorbid with insomnia [43]. In contrast, neural pathways in the vlPAG are critically implicated in stress-induced recovery sleep. For example, noradrenergic neurons in the vlPAG have been shown to promote NREM sleep via GABAergic projections to arousal-promoting nuclei, directly linking brainstem sleep circuits and stress [44]. Altogether, these findings suggest that dysfunction across multiple brain regions can induce insomnia. Further studies on dynamic interactions between these neural circuits are required to fully elucidate the mechanisms underlying quercetin-induced precise therapeutic effects on insomnia.

The results of this study showed that quercetin facilitated a compensatory increase in NREM sleep during the early recovery phase (1–6 h) post-ASD, whereas in the late recovery phase (19–24 h), it promoted normalization of the sleep-wake cycle. Notably, the compensatory increase in NREM sleep was primarily concentrated in the early recovery phase. Importantly, NREM sleep contributes to synaptic pruning, metabolic waste clearance, and overall bodily recovery [45]. These results suggest that quercetin-mediated regulation of NREM sleep during the early recovery phase aligns with the physiological demands of the body and provides important support for restoring physiological homeostasis.

SD-induced neuroinflammation in the brain has been characterized by the upregulation of inflammatory factors, such as IL-6, IL-1 β , TNF- α , cyclooxygenase-2, inducible NO synthase, sirtuin 6, and high mobility group box 1, in the mPFC of mice [46]. A recent study showed that SD causes region-specific changes in microglial density and morphology in brain regions such as the NAc, hippocampus, hypothalamus, mPFC, and thalamus in young mice. Among these, the highest density of Iba1-positive cells was found in the NAc, whereas hippocampus and mPFC exhibited the greatest total length and average number of microglia [5]. The increased microglial expression in the mPFC and NAc observed in these studies is consistent with the findings of the present study, underscoring the critical role of the mPFC and NAc in SD-induced neuroinflammation.

A key aspect warranting detailed discussion is that quercetin exerts sleep-regulating effects by attenuating neuroinflammation within specific brain regions. Recent studies report that quercetin exerts anti-inflammatory effects in neurodegenerative diseases by modulating inflammatory cytokine levels in the brain [47,48]. Furthermore, quercetin can cross the BBB and is widely distributed across the cerebral cortex and limbic system. A previous study confirmed that quercetin mitigated sodium fluoride-induced structural and functional damage to the rat mPFC by enhancing antioxidant capacity and inhibiting apoptosis, offering a potential strategy for protecting the CNS from neurotoxicity [49]. However, direct evidence for the anti-inflammatory effect of quercetin within the NAc remains limited. There-

fore, the observed reduction of microglia in the NAc in the present study may be attributed to quercetin-mediated suppression of inflammation in the mPFC, which then reduced the transmission of pro-inflammatory signals to the NAc, thereby alleviating local neuroinflammation.

c-Fos, a key marker of neuronal activation, plays a critical role in assessing neural activity in sleep-regulatory brain regions. This study showed that quercetin inhibited c-Fos expression in the mPFC, PVT, and BLA, while increasing its expression in the BNST, compared with that in the control group. This pattern needs to be further explored to elucidate underlying mechanisms. As a key component of the extended amygdala, the BNST is closely linked with high-arousal behavioral states. It is a highly heterogeneous region, exhibiting extensive diversity in cell types, with notable variations in spatial organization, molecular expression, synaptic inputs, axonal outputs, and functional regulation [50]. Various subtypes of BNST neurons project to brain regions essential for sleep-wake regulation, including the lateral hypothalamus (LH), ventral tegmental area, and arcuate nucleus [50]. Notably, optogenetic activation of BNST GABAergic neurons in mice has been shown to promote rapid transitions from NREM sleep to wakefulness; these neurons are also highly active during wakefulness and REM sleep [51]. Studies have confirmed bidirectional functional connectivity between the LH and BNST [52,53]. Additionally, excitatory BNST projections have been reported to activate REM-active neurons in the sublaterodorsal nucleus of the brainstem, contributing to REM sleep regulation [54]. Notably, quercetin treatment further increased c-Fos expression in the BNST in this study, suggesting that its effects may involve the neurochemical and cellular heterogeneity of the BNST. Future studies need to focus on integrating EEG-EMG and fiber photometry recordings, along with two-photon imaging, to monitor quercetin-induced sleep regulation in the cell-type-specific BNST neurons.

In contrast to its pronounced efficacy in ASD models, quercetin exhibited limited effectiveness in restoring post-CSD sleep homeostasis, showing only a reduction in REM sleep during the early recovery phase. ASD mainly impairs attention and working memory, along with inducing transient oxidative stress in the hippocampus and prefrontal cortex [55]. Mechanistically, ASD transiently activates the HPA axis and triggers microglial activation, leading to localized inflammation without systemic spread. In contrast, CSD causes “multi-target, irreversible” damage. Prolonged overactivation of the HPA axis results in a “depleted state”, accompanied by dysregulation of multiple neurotransmitter systems [4]. Reportedly, a study on rodents has shown that CSD disrupts BBB integrity, exacerbates microglial activation, and elevates proinflammatory cytokine levels (such as $\text{TNF-}\alpha$ and $\text{IL-1}\beta$) in the brain compared with the effects of ASD [56]. In the present study, quercetin displayed a tendency to enhance the restoration of post-ASD sleep

homeostasis but exerted only a weak effect in maintaining sleep homeostasis during CSD. This discrepancy may be attributable to the single-dose administration design used in this study. To overcome this limitation and facilitate clinical translation, future experiments should focus on identifying suitable delivery methods and optimizing quercetin dosage regimens to effectively regulate sleep homeostasis in various sleep disorder models.

5. Conclusion

The findings of this study reveal the potential of quercetin in promoting NREM sleep under basal conditions and post-ASD sleep homeostasis restoration, without influencing sleep pressure. The underlying mechanisms may involve inhibiting neuroinflammation and excessive neuronal activation in specific sleep-regulatory brain regions. These findings provide novel evidence supporting the role of natural compounds in regulating sleep and neuroimmune interactions, along with offering quercetin as a promising candidate for treating acute sleep disorders. Future research needs to aim to refine quercetin administration protocols, elucidate region-specific molecular mechanisms, and develop formulations with improved bioavailability, thereby laying a solid foundation for the clinical treatment of sleep disorders.

Availability of Data and Materials

The data during the current study are available from the corresponding author on reasonable request.

Author Contributions

RH and BW designed the research study. BW, ZH, and PF performed animal surgeries, sleep recordings, and immunohistochemistry. ZL, BN, and JZ analyzed EEG data and conducted statistical analyses. RH and BW wrote the initial draft. JZ offered experimental guidance. All authors contributed to editorial changes in the manuscript. All authors revised and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

All animal experiments were approved by the Institutional Animal Care and Use Committee of Xinxiang Medical University (IACUC Protocol No. XYLL-20240521), and we comply with all relevant ethical regulations. Every effort was made to minimize the number of animals used and reduce their suffering throughout the study. This study was conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals (8th edition, National Institutes of Health, USA). All protocols were designed to adhere to the 3Rs principles (Replacement, Reduction, Refinement) of animal research and were periodically reviewed by the IACUC to ensure ongoing compliance with ethical standards.

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Conflict of Interest

The authors declare no conflict of interest.

References

- [1] Feuth T. Interactions between sleep, inflammation, immunity and infections: A narrative review. *Immunity, Inflammation and Disease*. 2024; 12: e70046. <https://doi.org/10.1002/iid3.70046>.
- [2] Hu M, Shu X, Feng H, Xiao LD. Sleep, inflammation and cognitive function in middle-aged and older adults: A population-based study. *Journal of Affective Disorders*. 2021; 284: 120–125. <https://doi.org/10.1016/j.jad.2021.02.013>.
- [3] Flemming A. Sleep deprivation whips up cytokine storm. *Nature Reviews. Immunology*. 2024; 24: 2. <https://doi.org/10.1038/s41577-023-00980-9>.
- [4] Wright KP, Jr, Drake AL, Frey DJ, Fleshner M, Desouza CA, Gronfier C, *et al*. Influence of sleep deprivation and circadian misalignment on cortisol, inflammatory markers, and cytokine balance. *Brain, Behavior, and Immunity*. 2015; 47: 24–34. <https://doi.org/10.1016/j.bbi.2015.01.004>.
- [5] Ni RJ, Wang YY, Pu WJ, Wei YY, Wei JX, Zhao LS, *et al*. Differential effects of sleep deprivation on behavior and microglia in a brain-region-specific manner in young and aged male mice. *Brain, Behavior, and Immunity*. 2024; 117: 12–19. <https://doi.org/10.1016/j.bbi.2023.12.031>.
- [6] Ma C, Li B, Silverman D, Ding X, Li A, Xiao C, *et al*. Microglia regulate sleep through calcium-dependent modulation of norepinephrine transmission. *Nature Neuroscience*. 2024; 27: 249–258. <https://doi.org/10.1038/s41593-023-01548-5>.
- [7] Hristovska I, Robert M, Combet K, Honnorat J, Comte JC, Pascual O. Sleep decreases neuronal activity control of microglial dynamics in mice. *Nature Communications*. 2022; 13: 6273. <https://doi.org/10.1038/s41467-022-34035-9>.
- [8] Nebeling FC, Poll S, Justus LC, Steffen J, Keppler K, Mittag M, *et al*. Microglial motility is modulated by neuronal activity and correlates with dendritic spine plasticity in the hippocampus of awake mice. *eLife*. 2023; 12: e83176. <https://doi.org/10.7554/eLife.83176>.
- [9] Gu X, Zhao Z, Chen X, Zhang L, Fang H, Zhao T, *et al*. Imaging microglia surveillance during sleep-wake cycles in freely behaving mice. *eLife*. 2023; 12: RP86749. <https://doi.org/10.7554/eLife.86749>.
- [10] Uzategui NL, Güçer S, Richter C, Speidel A, Zirdum E, Duszenko M, *et al*. Live imaging of microglia during sleeping sickness reveals early and heterogeneous inflammatory responses. *Frontiers in Immunology*. 2023; 14: 1253648. <https://doi.org/10.3389/fimmu.2023.1253648>.
- [11] Li Y, Yao J, Han C, Yang J, Chaudhry MT, Wang S, *et al*. Quercetin, Inflammation and Immunity. *Nutrients*. 2016; 8: 167. <https://doi.org/10.3390/nu8030167>.
- [12] Jiang Y, Xie G, Alimujiang A, Xie H, Yang W, Yin F, *et al*. Protective Effects of Quercetin against MPP+-Induced Dopaminergic Neurons Injury via the Nrf2 Signaling Pathway. *Frontiers in Bioscience (Landmark Edition)*. 2023; 28: 42. <https://doi.org/10.31083/j.fbl2803042>.
- [13] Wu Y, Tang Z, Du S, Dai J, Shen J, Niu H, *et al*. Oral quercetin nanoparticles in hydrogel microspheres alleviate high-altitude sleep disturbance based on the gut-brain axis. *International Journal of Pharmaceutics*. 2024; 658: 124225. <https://doi.org/10.1016/j.ijpharm.2024.124225>.
- [14] Sun Y, Zhang H, Wu Z, Yu X, Yin Y, Qian S, *et al*. Quercitrin Rapidly Alleviated Depression-like Behaviors in Lipopolysaccharide-Treated Mice: The Involvement of PI3K/AKT/NF- κ B Signaling Suppression and CREB/BDNF Signaling Restoration in the Hippocampus. *ACS Chemical Neuroscience*. 2021; 12: 3387–3396. <https://doi.org/10.1021/acschemneuro.1c00371>.
- [15] Shen P, Lin W, Deng X, Ba X, Han L, Chen Z, *et al*. Potential Implications of Quercetin in Autoimmune Diseases. *Frontiers in Immunology*. 2021; 12: 689044. <https://doi.org/10.3389/fimmu.2021.689044>.
- [16] Wu J, Lv T, Liu Y, Liu Y, Han Y, Liu X, *et al*. The role of quercetin in NLRP3-associated inflammation. *Inflammopharmacology*. 2024; 32: 3585–3610. <https://doi.org/10.1007/s10787-024-01566-0>.
- [17] Ogrodnik M, Evans SA, Fielder E, Victorelli S, Kruger P, Salmonowicz H, *et al*. Whole-body senescent cell clearance alleviates age-related brain inflammation and cognitive impairment in mice. *Aging Cell*. 2021; 20: e13296. <https://doi.org/10.1111/acer.13296>.
- [18] Zhang Y, Xie Y, Cheng Z, Xi K, Huang X, Kuang F, *et al*. Quercetin ameliorates memory impairment by inhibiting abnormal microglial activation in a mouse model of paradoxical sleep deprivation. *Biochemical and Biophysical Research Communications*. 2022; 632: 10–16. <https://doi.org/10.1016/j.bbrc.2022.09.088>.
- [19] Zhao W, Wang J, Bi W, Ferruzzi M, Yemul S, Freire D, *et al*. Novel application of brain-targeting polyphenol compounds in sleep deprivation-induced cognitive dysfunction. *Neurochemistry International*. 2015; 89: 191–197. <https://doi.org/10.1016/j.neuint.2015.07.023>.
- [20] Bappi MH, Mia MN, Ansari SA, Ansari IA, Prottay AAS, Akbor MS, *et al*. Quercetin increases the antidepressant-like effects of sclareol and antagonizes diazepam in thiopental sodium-induced sleeping mice: A possible GABAergic transmission intervention. *Phytotherapy Research: PTR*. 2024; 38: 2198–2214. <https://doi.org/10.1002/ptr.8139>.
- [21] Kambe D, Kotani M, Yoshimoto M, Kaku S, Chaki S, Honda K. Effects of quercetin on the sleep-wake cycle in rats: involvement of gamma-aminobutyric acid receptor type A in regulation of rapid eye movement sleep. *Brain Research*. 2010; 1330: 83–88. <https://doi.org/10.1016/j.brainres.2010.03.033>.
- [22] Lasure VU, Singh Gautam A, Singh RK. Quercetin ameliorates neuroinflammatory and neurodegenerative biomarkers in

- the brain and improves neurobehavioral parameters in a repeated intranasal amyloid-beta exposed model of Alzheimer's disease. *Food & Function*. 2024; 15: 8712–8728. <https://doi.org/10.1039/d4fo02602k>.
- [23] Li R, Pan Y, Jing N, Wang T, Shi Y, Hao L, *et al.* Flavonoids from mulberry leaves exhibit sleep-improving effects via regulating GABA and 5-HT receptors. *Journal of Ethnopharmacology*. 2025; 337: 118734. <https://doi.org/10.1016/j.jep.2024.118734>.
- [24] Calil Brondani J, Reginato FZ, da Silva Brum E, de Souza Vençato M, Lima Lhamas C, Viana C, *et al.* Evaluation of acute and subacute toxicity of hydroethanolic extract of *Dolichandra unguis-cati* L. leaves in rats. *Journal of Ethnopharmacology*. 2017; 202: 147–153. <https://doi.org/10.1016/j.jep.2017.03.011>.
- [25] Qu W, Deng X, Guo T, Zhou C, Ma H. Enhancing the encapsulation efficiency, bioavailability, and antioxidant efficacy of quercetin and maize peptide by a novel multi-frequency pulsed ultrasonic-assisted nanoliposomal encapsulation system. *Food Research International* (Ottawa, Ont.). 2025; 218: 116874. <https://doi.org/10.1016/j.foodres.2025.116874>.
- [26] Abdelrazek DA, Ibrahim MA, Hassan NH, Hassanien EI, Farroh KY, Abass HI. Neuroprotective effect of quercetin and nano-quercetin against cyclophosphamide-induced oxidative stress in the rat brain: Role of Nrf2/HO-1/Keap-1 signaling pathway. *Neurotoxicology*. 2023; 98: 16–28. <https://doi.org/10.1016/j.neuro.2023.06.008>.
- [27] Agrawal M, Saraf S, Saraf S, Antimisariis SG, Chougule MB, Shoyele SA, *et al.* Nose-to-brain drug delivery: An update on clinical challenges and progress towards approval of anti-Alzheimer drugs. *Journal of Controlled Release: Official Journal of the Controlled Release Society*. 2018; 281: 139–177. <https://doi.org/10.1016/j.jconrel.2018.05.011>.
- [28] Liu Y, Zhao D, Yang F, Ye C, Chen Z, Chen Y, *et al.* In Situ Self-Assembled Phytopolyphenol-Coordinated Intelligent Nanotherapeutics for Multipronged Management of Ferroptosis-Driven Alzheimer's Disease. *ACS Nano*. 2024; 18: 7890–7906. <https://doi.org/10.1021/acsnano.3c09286>.
- [29] Borbély A. The two-process model of sleep regulation: Beginnings and outlook. *Journal of Sleep Research*. 2022; 31: e13598. <https://doi.org/10.1111/jsr.13598>.
- [30] Brown RE, Basheer R, McKenna JT, Strecker RE, McCarley RW. Control of sleep and wakefulness. *Physiological Reviews*. 2012; 92: 1087–1187. <https://doi.org/10.1152/physrev.00032.2011>.
- [31] Borbély AA, Achermann P. Sleep homeostasis and models of sleep regulation. *Journal of Biological Rhythms*. 1999; 14: 557–568. <https://doi.org/10.1177/074873099129000894>.
- [32] Besedovsky L, Lange T, Haack M. The Sleep-Immune Crosstalk in Health and Disease. *Physiological Reviews*. 2019; 99: 1325–1380. <https://doi.org/10.1152/physrev.00010.2018>.
- [33] Scammell TE, Arrigoni E, Lipton JO. Neural Circuitry of Wakefulness and Sleep. *Neuron*. 2017; 93: 747–765. <https://doi.org/10.1016/j.neuron.2017.01.014>.
- [34] Cirelli C. The genetic and molecular regulation of sleep: from fruit flies to humans. *Nature Reviews. Neuroscience*. 2009; 10: 549–560. <https://doi.org/10.1038/nrn2683>.
- [35] Brown RE, McKenna JT, Winston S, Basheer R, Yanagawa Y, Thakkar MM, *et al.* Characterization of GABAergic neurons in rapid-eye-movement sleep controlling regions of the brainstem reticular formation in GAD67-green fluorescent protein knock-in mice. *The European Journal of Neuroscience*. 2008; 27: 352–363. <https://doi.org/10.1111/j.1460-9568.2008.06024.x>.
- [36] Kanazawa LKS, Vecchia DD, Wendler EM, Hocayen PDAS, Dos Reis Lívoro FA, Stipp MC, *et al.* Quercetin reduces manic-like behavior and brain oxidative stress induced by paradoxical sleep deprivation in mice. *Free Radical Biology & Medicine*. 2016; 99: 79–86. <https://doi.org/10.1016/j.freeradbiomed.2016.07.027>.
- [37] Lou T, Ma J, Wang Z, Terakoshi Y, Lee CY, Asher G, *et al.* Hyper-Activation of mPFC Underlies Specific Traumatic Stress-Induced Sleep-Wake EEG Disturbances. *Frontiers in Neuroscience*. 2020; 14: 883. <https://doi.org/10.3389/fnins.2020.00883>.
- [38] McCullough KM, Missig G, Robble MA, Foilb AR, Wells AM, Hartmann J, *et al.* Nucleus Accumbens Medium Spiny Neuron Subtypes Differentially Regulate Stress-Associated Alterations in Sleep Architecture. *Biological Psychiatry*. 2021; 89: 1138–1149. <https://doi.org/10.1016/j.biopsych.2020.12.030>.
- [39] Li M, Li W, Liang S, Liao X, Gu M, Li H, *et al.* BNST GABAergic neurons modulate wakefulness over sleep and anesthesia. *Communications Biology*. 2024; 7: 339. <https://doi.org/10.1038/s42003-024-06028-5>.
- [40] Hasegawa E, Miyasaka A, Sakurai K, Cherasse Y, Li Y, Sakurai T. Rapid eye movement sleep is initiated by basolateral amygdala dopamine signaling in mice. *Science (New York, N.Y.)*. 2022; 375: 994–1000. <https://doi.org/10.1126/science.abl6618>.
- [41] Sanford LD, Adkins AM, Wellman LL. The Amygdala as a Mediator of Sleep and Emotion in Normal and Disordered States. *Journal of Integrative Neuroscience*. 2022; 21: 163. <https://doi.org/10.31083/j.jin2106163>.
- [42] Zhao J, Liu C, Zhang F, Zheng Z, Luo F, Xia J, *et al.* A paraventricular thalamus to central amygdala neural circuit modulates acute stress-induced heightened wakefulness. *Cell Reports*. 2022; 41: 111824. <https://doi.org/10.1016/j.celrep.2022.111824>.
- [43] Liu D, Wang Q, Li Y, Yuan Z, Liu Z, Guo J, *et al.* Fructus garciniae ameliorates anxiety-like behaviors induced by sleep deprivation via regulating hippocampal metabolomics and gut microbiota. *Frontiers in Cellular and Infection Microbiology*. 2023; 13: 1167312. <https://doi.org/10.3389/fcimb.2023.1167312>.
- [44] Zhong P, Zhang Z, Barger Z, Ma C, Liu D, Ding X, *et al.* Control of Non-REM Sleep by Midbrain Neurotensinergic Neurons. *Neuron*. 2019; 104: 795–809.e6. <https://doi.org/10.1016/j.neuron.2019.08.026>.
- [45] Fultz NE, Bonmassar G, Setsompop K, Stickgold RA, Rosen BR, Polimeni JR, *et al.* Coupled electrophysiological, hemodynamic, and cerebrospinal fluid oscillations in human sleep. *Science (New York, N.Y.)*. 2019; 366: 628–631. <https://doi.org/10.1126/science.aax5440>.
- [46] Cao D, Zhao Y, Wang Y, Wei D, Yan M, Su S, *et al.* Effects of sleep deprivation on anxiety-depressive-like behavior and neuroinflammation. *Brain Research*. 2024; 1836: 148916. <https://doi.org/10.1016/j.brainres.2024.148916>.
- [47] Wang L, Sun J, Miao Z, Jiang X, Zheng Y, Yang G. Quercitrin improved cognitive impairment through inhibiting inflammation induced by microglia in Alzheimer's disease mice. *Neuroreport*. 2022; 33: 327–335. <https://doi.org/10.1097/WNR.0000000000001783>.
- [48] Sharma S, Raj K, Singh S. Neuroprotective Effect of Quercetin in Combination with Piperine Against Rotenone- and Iron Supplement-Induced Parkinson's Disease in Experimental Rats. *Neurotoxicity Research*. 2020; 37: 198–209. <https://doi.org/10.1007/s12640-019-00120-z>.
- [49] Javanbakht P, Talebinasab A, Asadi-Golshan R, Shabani M, Kashani IR, Mojaverrostami S. Effects of Quercetin against fluoride-induced neurotoxicity in the medial prefrontal cortex of rats: A stereological, histochemical and behavioral study. *Food and Chemical Toxicology: an International Journal Published for the British Industrial Biological Research Association*. 2025; 196: 115126. <https://doi.org/10.1016/j.fct.2024.115126>.
- [50] Giardino WJ, Pomrenze MB. Extended Amygdala Neuropeptide Circuitry of Emotional Arousal: Waking Up on the Wrong Side

- of the Bed Nuclei of Stria Terminalis. *Frontiers in Behavioral Neuroscience*. 2021; 15: 613025. <https://doi.org/10.3389/fnbeh.2021.613025>.
- [51] Kodani S, Soya S, Sakurai T. Excitation of GABAergic Neurons in the Bed Nucleus of the Stria Terminalis Triggers Immediate Transition from Non-Rapid Eye Movement Sleep to Wakefulness in Mice. *The Journal of Neuroscience: the Official Journal of the Society for Neuroscience*. 2017; 37: 7164–7176. <https://doi.org/10.1523/JNEUROSCI.0245-17.2017>.
- [52] Peyron C, Tighe DK, van den Pol AN, de Lecea L, Heller HC, Sutcliffe JG, *et al.* Neurons containing hypocretin (orexin) project to multiple neuronal systems. *The Journal of Neuroscience: the Official Journal of the Society for Neuroscience*. 1998; 18: 9996–10015. <https://doi.org/10.1523/JNEUROSCI.18-23-09996.1998>.
- [53] González JA, Iordanidou P, Strom M, Adamantidis A, Burdakov D. Awake dynamics and brain-wide direct inputs of hypothalamic MCH and orexin networks. *Nature Communications*. 2016; 7: 11395. <https://doi.org/10.1038/ncomms11395>.
- [54] Rodrigo-Angulo ML, Heredero S, Rodríguez-Veiga E, Reinoso-Suárez F. GABAergic and non-GABAergic thalamic, hypothalamic and basal forebrain projections to the ventral oral pontine reticular nucleus: their implication in REM sleep modulation. *Brain Research*. 2008; 1210: 116–125. <https://doi.org/10.1016/j.brainres.2008.02.095>.
- [55] Curcio G, Ferrara M, De Gennaro L. Sleep loss, learning capacity and academic performance. *Sleep Medicine Reviews*. 2006; 10: 323–337. <https://doi.org/10.1016/j.smrv.2005.11.001>.
- [56] Fahmawi A, Khalifeh MS, Alzoubi KH, Rababa'h AM. The Effects of Acute and Chronic Sleep Deprivation on the Immune Profile in the Rat. *Current Molecular Pharmacology*. 2023; 16: 101–108. <https://doi.org/10.2174/1874467215666220316104321>.