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# Consequences of sleep deprivation

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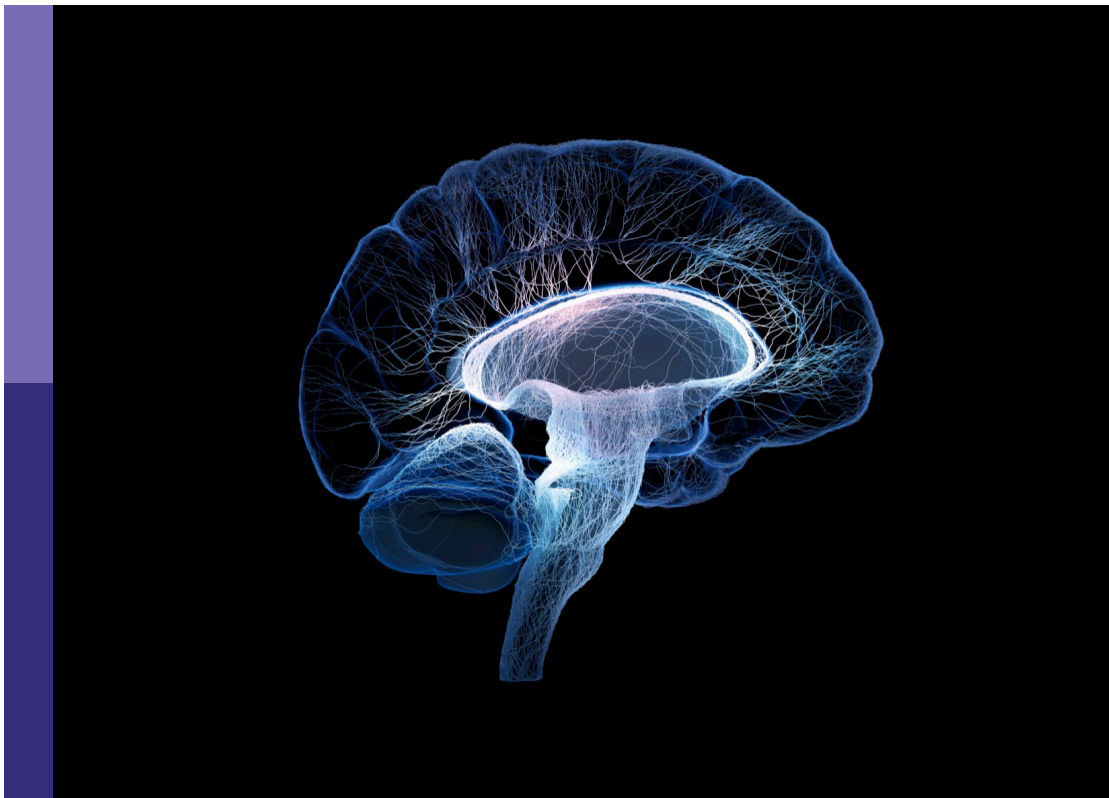

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# Consequences of sleep deprivation

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# Editorial: Consequences of sleep deprivation

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Editorial on the Research Topic
Consequences of sleep deprivation

Sleep deprivation occurs when an individual consistently fails to obtain an adequate amount of sleep, either due to external factors or internal disruptions. Sleep deprivation is a pervasive problem that affects millions of people worldwide. In our fast-paced and demanding modern society, where productivity is often prioritized over rest, the value of a good night's sleep has been overlooked and downplayed. Karen Russell, the acclaimed author of the dystopian novel "*Sleep donation*", envisaged a near future with hundreds of thousands of cases suffering from an terminal insomnia crisis, where sleep is a commodity which can be donated to the lucky few (Russell, 2014). While the ability to donate sleep to others appears far-fetched at present, it is clear in the scientific community that the consequences of sleep deprivation can be far-reaching, impacting not only our physical health but also our mental wellbeing and cognitive abilities. Thus, this Research Topic which focuses on the mechanisms, consequences and biomarkers of sleep deprivation is important and timely and can inform the development of novel brain stimulation or pharmacological treatments to prevent accidents and ameliorate neuropsychiatric disorders involving sleep disruption (Brown et al., 2022). For instance, findings that increased orexin levels in patients Alzheimer's disease are linked to both sleep disruption and cognitive impairment (Liguori et al., 2014) suggest that the use of orexin receptor antagonists could be beneficial.

Sleep disruption affects virtually all physiological functions. Roach et al., using sophisticated molecular/genetic tools in a Drosophila model, identified specific "clock" neurons that are modulated by sleep disruption to change temperature preference. Interestingly, while sleep deprivation, sleep fragmentation and a social jetlag protocol led to a change in temperature preference, only the sleep deprivation protocol impaired memory formation suggesting that temperature preference is a more sensitive indicator of sleep disruption than learning and memory and a potential biomarker. The relationship between sleep and memory was evident from the 1st century when the orator Quintilian stated ≪*...quae statim referri non poterant, contexuntur postera die, confirmatque memoriam idem illud tempus quod esse in causa solet oblivionis...*≫, "What may seem unattainable initially can be effortlessly achieved the following day, and the time that is commonly thought to induce forgetfulness (i.e., sleep) is discovered to enhance memory" (Quintilian). Two millennia later, in 1924, Jenkins and Dallenback documented the first experimental evidence of the sleep effect preventing the normal memory decay curve (Jenkins and Dallenbach, 1924). Whitney et al. revised narratively the generally accepted approach to analyzing effects of sleep deprivation on subsequent memory and learning by

means of its effects on encoding. The authors suggested an intriguing framework with which to understand sleep loss and memory in terms of temporary amnesia from sleep loss (TASL). The view of the TASL framework is that amnesia and the amnesia-like deficits observed during sleep deprivation not only affect memory processes but will also be apparent in cognitive processes that rely on those memory processes, such as decision-making. The hippocampus, interacting with higher structures, such as the prefrontal cortex, to produce complex cognition and behavioral performance, is compromised by sleep disruption. Li B. et al. used resting-state functional magnetic resonance imaging (fMRI) to study the relationship between the changes of the precuneus (PC) functional connectivity and alertness decline after total sleep deprivation (SD). SD induced decreased functional connectivity between the right PC and the right middle frontal gyrus (MFG). Moreover, there was a significant correlation between the decreased PC functional connectivity and alertness decline after total SD. They hypothesized that the interruption of the connection between the right PC and the right MFG is related to the observed decline in alert attention after acute SD. These results provide further indications of the changes in cortical circuits which underlie cognitive impairments during SD. Functional connectivity changes also occur in primary insomnia. Xie et al. examined the abnormal resting state functional connections (RSFCs) in patients with insomnia. The authors found structural changes in the right middle frontal gyrus and right inferior frontal gyrus accompanied by RSFC changes. Thus, these brain regions may represent potential targets for non-invasive brain stimulation treatments (Brown et al., 2022). Continuing the cognitive theme Wu et al., evaluated neurocognitive disorders including postoperative delirium and cognitive decline in the early postoperative period in patients with or without excessive daytime sleepiness who had a moderate to high risk of obstructive sleep apnea (OSA). The authors found a significant correlation with postoperative cognitive decline for those OSA patients who had excessive daytime sleepiness, a potentially treatable phenotype.

Two studies by Peng and colleagues investigated the effect of total SD (TSD) on evoked potentials and working memory. The first study from Peng, Dai et al. found that TSD can impair working memory capacity, which is characterized by lower amplitude and prolonged latency of working-memory related N2-P3 ERPs components. In a second study Peng, Hou et al. utilized ERPs to investigate the restorative effects of 8 h of recovery sleep on working memory impairments induced by total sleep deprivation for 36 h. Eight hours of recovery sleep attenuated the decrease in working memory performance caused by 36 h of TSD. The authors hypothesized that these restorative effects are likely to occur during SWS. However, RS had limited effects and further studies should establish whether 8 h of RS can restore cognitive function to baseline levels.

In our 24/7 society a generally held presumption has been that while chronic sleep disruption results in neurobehavioral impairments, performance deficits are reversed with limited-period recovery sleep (e.g., over the weekend) (Zamore and Veasey, 2022). Li C. et al., studied the effects of 52 h of SD and of 14 h of recovery sleep on resting-state fMRI and the availability of A1 adenosine receptors using a PET scan. The authors found negative correlations between A1AR availability and BOLD activity in the left superior/middle temporal gyrus and left postcentral gyrus of the human brain providing new insights into the molecular basis of neuronal responses induced by high homeostatic sleep pressure. The relationship between sleep and pain is complex and bidirectional. Sleep deprivation can exacerbate low back pain by lowering the pain threshold and increasing sensitivity to pain (Alsaadi et al., 2014), Luo et al. showed in an analysis of a large GWAS dataset that there was a mutual causal relationship between genetic variants which affect sleep and low back pain, therefore sleep regulators should be considered in a more comprehensive management of pain, if this observation will confirmed by prospective studies. Alterations of sleep continuity negatively affect synaptic plasticity in the hippocampus (Tartar et al., 2006) but less attention has been directed to the effects of sleep disruption in other parts of the nervous system. Han et al. evaluated how sleep deprivation (SD) affects the process of olfactory sensory neurons (OSN) regeneration following olfactory epithelium injury. SD accompanied by disturbed circadian activity could induce structurally negative effects on OSN regeneration, preferentially in the dorsomedial area of the OE, and that this area-specific regeneration delay might involve the biological activity of neurons expressing NQO1 (quinone dehydrogenase 1). Therefore, circadian activity modulates adult neurogenesis supporting the hypothesis of relationship with neurodegenerative diseases as well as olfactory disorders.

Increasing evidence suggests a role for the immune system and inflammatory responses in both normal sleep regulation and disrupted sleep (Irwin, 2019). The review by Amini et al. investigates whether there is a causal relationship between SD and the NLRP3 inflammasome, a key component of the innate immune responses, or its downstream pathways. They conclude that indeed the NLRP3 inflammasome is a potential target for therapy in order to improve the clinical outcomes of SD.

The 24/7 society is convinced that sleep will become increasingly more flexible and reduced sleep time will allow for greater personal freedom and success, until there is no difference between day and night, light and dark, and action and repose (Crary, 2013). This point of view is contrary to the findings of sleep and circadian science, which show that sleep disruption is detrimental to productivity increases accidents and impairs both physical and mental health (Czeisler, 2006). The 12 articles in this Research Topic illustrate the breadth of research on this topic, revealing the effects of SD on attention, working memory, temperature preference, pain and neural plasticity and some of the potential molecular mediators and brain circuits which are affected. Furthermore, they suggest that treatment of sleep disruption could have beneficial effects in the treatment of post-operative cognitive disorders and pain. We hope the readers will find them useful and interesting.

## Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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## Conflict of interest

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ORIGINAL RESEARCH
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# Effect of Sleep Deprivation on the Working Memory-Related N2-P3 Components of the Event-Related Potential Waveform

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Working memory is very sensitive to acute sleep deprivation, and many studies focus on the brain areas or network activities of working memory after sleep deprivation. However, little is known about event-related potential (ERP)-related changes in working memory after sleep loss. The purpose of this research was to explore the effects of 36 h of total sleep deprivation (TSD) on working memory through ERPs. Sixteen healthy college students performed working memory tasks while rested and after 36 h of TSD, and electroencephalography (EEG) data were simultaneously recorded while the subjects completed working memory tasks that included different types of stimulus materials. ERP data were statistically analyzed using repeated measurements analysis of variance to observe the changes in the working memory-related N2-P3 components. Compared with baseline before TSD, the amplitude of N2-P3 components related to working memory decreased, and the latency was prolonged after TSD. However, the increased amplitude of the P2 wave and the prolonged latency were found after 36 h of TSD. Thus, TSD can impair working memory capacity, which is characterized by lower amplitude and prolonged latency.

**Keywords: sleep deprivation, working memory, event related potentials, electroencephalography, n-back**

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## INTRODUCTION

With the progress of society and changes in work rhythm, an increasing number of people are suffering from sleep deprivation. Sleep deprivation not only damages the physical and mental health of the individual but also seriously affects work performance, causing work errors and even accidents. Therefore, understanding the mechanism of sleep deprivation that affects cognitive function is of great significance for effectively preventing the effects of sleep deprivation.

Previous studies have revealed that sleep deprivation can cause a series of changes in an individual's mood, cognitive ability, work performance, and immune function (Choo et al., 2005). The lack of sleep disrupts body circulation and affects the cognitive and emotional abilities of individuals (Raymond, 1988). Several studies have revealed that sleep deprivation impairs response inhibition (Harrison and Horne, 1998; Muzur et al., 2002; Jennings et al., 2003). For example, after 36 h of sleep deprivation, the individual's ability to suppress negative stimuli decreased (Chuah et al., 2006). Neuroimaging studies have suggested that sleep deprivation reduces an individual's

low-level of visual processing ability (Anderson and Platten, 2011; Ning et al., 2014). In addition, sleep deprivation impairs the hippocampus and could affect memory by destroying synaptic plasticity (Cote et al., 2014). Thomas (2003) has indicated that lack of sleep reduced cerebral blood flow and metabolic rate in the thalamus, prefrontal cortex, and parietal cortex (Géraldine et al., 2005). Jarraya and colleagues found that partial sleep deprivation significantly affected neuropsychological functions such as verbal instant memory, attention, and alertness (Thomas, 2003). Furthermore, some studies have revealed that the cumulative effects of partial sleep deprivation could severely impair cognitive function and behavior (Van Dongen, 2004; Scott et al., 2006; Jarraya et al., 2013).

Working memory is a system that used to store and process information and which is a cognitive function with limited capacity (Bartel et al., 2004). Moreover, the information stored in the working memory system can be changed from short-term memory to long-term memory through retelling and other memory methods. Working memory is the transition between short-term and long-term memory systems, which is very pivotal in human message processing (Miyake and Shah, 1999). It provides a temporary storage space and the resources needed to process information, such as voice understanding, reasoning, and learning. Sleep deprivation has been shown to affect working memory first.

Previous studies have used the n-back working memory paradigm in participants who underwent sleep deprivation and found that lack of sleep induces a decrease in metabolic activity in the brain's regional network, which is mainly effected information processing and reaction inhibition (Baddeley, 2000; Zhang et al., 2019). Impaired working memory after sleep deprivation is related to the activation of the default network in tasks (Chee and Chuah, 2008), which may be related to the important role of the thalamus in cortical alertness. For instance, sleep deprivation increased the connection between the hippocampus, thalamus, and default network, which was often accompanied by higher subjective drowsiness and worse performance of working memory (Lei et al., 2015; Li et al., 2016). Studies on sleep deprivation identi?ed that increased latency and reduced amplitude of the P3 component were associated with prolonged sobriety (Morris et al., 1992; Jones and Harrison, 2001; Panjwani et al., 2010). The decrease in the P3 wave might reflect a decrease in participants' attention and a reduction in the discernment of target stimuli (Koslowsky and Babkoff, 1992).

However, few studies have provided electrophysiological evidence for impaired working memory after sleep deprivation. The n-back task is considered a common method to assess working memory (Owen et al., 2005; Jaeggi et al., 2010). Zhang et al. designed a two-back pronunciation working memory task to explore the decreased message alternate of working memory during sleep deprivation, but few studies have used different types of working memory tasks in a single experiment. In the present study, we designed different types of working memory tasks (pronunciation working memory, spatial working memory, and object working memory) to explore the impairment of cognitive function by TSD and recorded participant EEG data at 2 time points (baseline and 36 h-TSD). All of the tasks adopted a 2-back paradigm. This study evaluated the changes in the N2-P3 wave related to working memory during TSD and analyzed the temporal characteristics of the effects of sleep deprivation on working memory. Our findings provide experimental evidence for the effects of sleep deprivation on cognitive function.

# MATERIALS AND METHODS

## Participants

Sixteen young, healthy, right-handed male students participated in this study. We recruited participants by advertising on the campus. The participants all had good sleep habits (PSQI $<5$). All participants were aged between 21 and 28 years with an average age of 23 years, and none of the participants had any mental or physical illness. All participants had normal vision or corrected vision above 1.0 and intelligence scores $>110$ on the Raven Test. Before the experiment, the experimenter explained the procedure and points for attention to the participants to make sure they were familiar with the method and procedure. In the 2 weeks before the experiment, the participants slept regularly for 7–9 h per day, without smoking, drinking coffee, drinking alcohol, or consuming any medication for 2 days before the experiment. Before the experiment, all the participants provided written informed consent. The experimental scheme was approved by the Ethics Committee of the Fourth Military Medical University and Beihang University.

## Experimental Design

Three types of working memory tasks were presented to all participants. They were two-back pronunciation working memory task (see **Figure 1**), two-back spatial working memory task (see **Figure 2**), and two-back object working memory task (see **Figure 3**). The stimulate materials of the tasks were 15 case-insensitive English letters that excluding the ones with similar

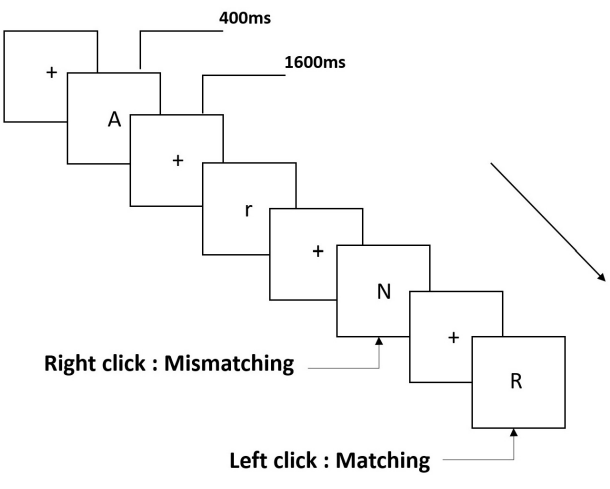


**FIGURE 1 |** Schematic diagram of the pronunciation working memory task.

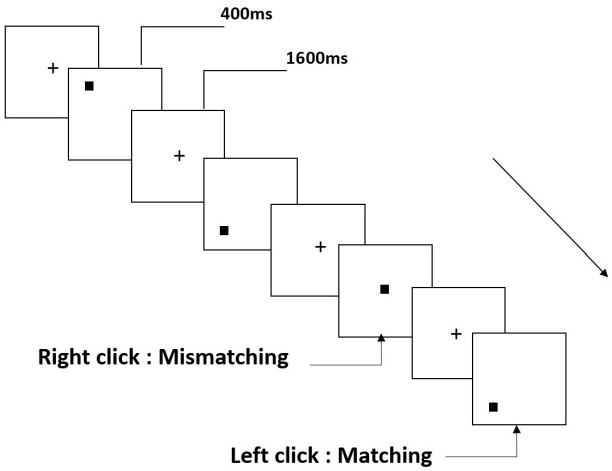


**FIGURE 2 |** Schematic diagram of the spatial working memory task.

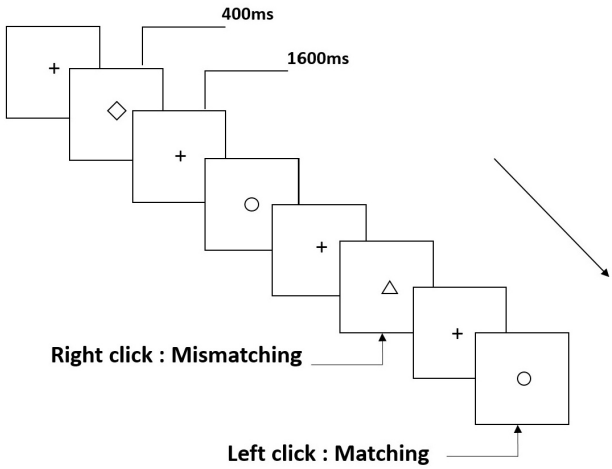


**FIGURE 3 |** Schematic diagram of the object working memory task.

letters, such as L/l, M/m; small black squares; and 12 geometric figures, respectively. All of the materials were shown in black color on a white background, with an approximate visual angle of 1.5° × 1.5° (width: 2.0 cm, height: 2.0 cm) subtending. 122 trails were comprised in each task and, in each trail, the target stimulus was presented for 400 ms two trails after the presentation of objective stimulus, with the 1,600 ms stimulus onset asynchrony time (SOA) that was marked by a white "+."The participants were asked to click the left mouse button when the target and objective stimulus were the same ("matching"), while click the right mouse button when they were not ("mismatching"). The matching or not condition were presented in a pseudorandom order with a 1:1 ratio.

## Experimental Procedures

Before the experiment, the participants were instructed of the experimental task. They were informed to practice the three types of working memory tasks until an accuracy rate of 90% was achieved. Participants visited the laboratory the day before the experiment and slept in the laboratory that night. The two partner participants performed the experiments at the same time. Three types of working memory tasks were performed at 7:30 am to 8:30 am the next morning with simultaneous electroencephalogram (EEG) recording (baseline). The second EEG recording (36 h-TSD) was conducted after a 36-h period during which the participants were not allowed to sleep. During the entire experiment time, central inhibition and stimulant drugs were forbidden. The participants were accompanied, observed and reminded by nursing staff in order to keep them awake throughout the TSD session.

## EEG Recordings

A continuous scalp EEG was recorded using electrode caps placed in 64 locations using the 10–20 system with a SynAmps2 amplifier. The bilateral mastoids (A1 and A2) were used for reference, and the forehead was used as a ground. EEGs were recorded at 1,000 Hz, and the impedance of all channels was maintained below 5 kΩ. Four additional electrodes were placed above and below the right and left eyes to record a bipolar vertical and horizontal electrooculogram.

## Data Analysis of Behavioral Experiments

Due to technical errors, two cases were deleted while other 14 cases were included in the following statistical analysis. Behavioral data included the mean reaction time, correct rate and the correct number per unit time. Behavioral data in baseline and 36 h-TSD states were recorded for analyzing. The analyses were run by IBM SPSS (V22.2), where the repeated measures analysis of variance (ANOVA) method with Greenhouse-Geisser was Bonferroni *post-hoc* analysis were launched. The statistical results were presented as the mean and standard deviation (SD).

## EEG Data Analysis

Scan 4.3 program was used to analyze the EEG data, where the EEG artifacts of the eye movement were corrected by ocular artifact reduction method. Epochs ranging from -100 to 800 ms of the continuous EEG data were extracted and filtered by a bandpass filter from 0.5 to 30 Hz with the frequency slope of 24 dB/oct. The trials in which the voltage exceeded ± 100 μV were rejected and the baseline was corrected to a mean amplitude of 100 ms. The EEG components were averaged and calculated with only the corrected responses. The ERP components P2 (100–250 ms), N2 (150–350 ms), and P3 (250–450 ms) of the stimulus trials were identified and quantified. The grand-average peak amplitudes and latencies of the N2 and P3 components were calculated separately at F3, Fz, F4, C3, Cz, C4, P3, Pz, and P4, and the P2 component was calculated at F3, Fz, F4, C3, Cz, and C4 (Casement et al., 2006; Verweij et al., 2014).

Repeated measures ANOVA was employed for all ERP results. The main effects and the interactions between sleep states (baseline and 36 h-TSD), tasks (pronunciation working memory, spatial working memory, and object working memory), regions (frontal, central, and parietal; the P2 component was analyzed only on the frontal and central regions), and sites

(left, middle, and right) were statistically analyzed employing repeated measures ANOVA, which included Greenhouse-Geisser corrections for non-sphericity and Bonferroni *post-hoc* tests.

# RESULTS

## Behavioral Performance

The results of the behavioral experiments are shown in **Table 1**. The mean reaction time was longer in the 36 h-TSD state than at baseline with a trend to increase [$F_{(1,13)} = 2.563$, $P = 0.133$] but without significant differences. ANOVA revealed that the correct rate of the task was significantly different between the baseline and 36 h-TSD [$F_{(1,13)} = 10.153$, $P = 0.007$]. The correct number per unit time showed a significant main effect of time during 36 h-TSD [$F_{(1,13)} = 7.010$, $P = 0.020$].

## Amplitude

Compared to the baseline, a significant decrease was observed in the amplitude of P3 [$F_{(1,13)} = 12.692$, $P = 0.003$], and a significant increase was observed in the amplitude of P2 [$F_{(1,13)} = 69.357$, $P = 0.000$] after TSD. Although the N2 amplitude decreased after 36 h of TSD, the difference did not reach statistical significance (**Table 2**).

Significant main effects of regions and sites on the P2 amplitude were found [$F_{(1,13)} = 15.889$, $P = 0.002$; $F_{(2,26)} = 26.190$, $P = 0.000$, respectively] under the TSD condition. During TSD, the maximum amplitude of P2 appeared in the frontal region (**Figure 4**). In addition, the differences in P2 amplitudes in different regions (frontal vs. central) were more significant [$F_{(2,26)} = 8.996$, $P = 0.001$] in the bilateral electrodes (left: $P = 0.001$; right: $P = 0.000$) than in the middle electrodes (**Figure 4**). A significant main effect of the region [$F_{(2,\ 26)} = 4.137$, $P = 0.050$] and site [$F_{(2,\ 26)} = 7.46$,$P = 0.003$] on N2 revealed that the N2 amplitude was more negative in the frontal than in the central region ($P = 0.008$, **Figure 4**) and was smaller on the right than on the left side ($P = 0.011$, **Figures 5A2,B2**). A main effect of site on the P3 amplitude was observed [$F_{(2,26)} = 5.363$, $P = 0.023$]. The amplitude of P3 was more positive in the middle than on the left side ($P = 0.009$, **Figure 4**). A significant interaction effect between time and region was observed for the P3 amplitude [$F_{(2,26)} = 7.375$, $P = 0.012$]. During TSD, the reduction in P3 amplitude was more significant in the frontal and central regions than in the parietal region ($P = 0.005$; $P = 0.003$) (**Figure 5C3**). No other main effects or interaction effects reached statistical significance.

**TABLE 1 |** Performance data (mean ± SD) on the 2-back task at baseline and after 36 h-TSD.

| | Baseline | 36 h-TSD |
|---|---|---|
| Mean Reaction time (ms) | 507.26 (82.04) | 542.77 (103.73) |
| Correct rate (%) | 0.94 (0.04) | 0.85 (0.12)* |
| Correct number/sec | 1.91 (0.35) | 1.64 (0.42)* |

*$P < 0.05$, vs. baseline.*

## Latency

The latencies of N2 [$F_{(1,\ 13)} = 6.673$, $P = 0.023$] and P2 [$F_{(1,13)} = 8.439$, $P = 0.012$] were significantly prolonged after TSD. Although the P3 latency was prolonged after 36 h of TSD, the difference did not reach statistical significance (**Table 3**).

The significant main effect of region on N2 [$F_{(2,\ 26)} = 13.789$, $P = 0.001$] and P3 [$F_{(2,\ 26)} = 45.226$, $P = 0.000$] revealed that the latency of the N2-P3 components was shorter in the parietal region than in the frontal region ($P = 0.002$; $P = 0.000$) and central region ($P = 0.000$; $P = 0.000$) (**Figure 4**). The latency of the P3 wave was significantly longer on the left side than on the right side [$F_{(2,26)} = 8.812$, $P = 0.001$] (**Figure 4**).

No other main effects or interaction effects reached statistical significance.

The N2, P2, and P3 amplitudes and latencies that were elicited at the nine electrode sites are presented in **Figure 4**. The topographic map of the correct response in the working memory task in different sleep conditions (baseline, 36 h-TSD and the difference between the two conditions) is presented in **Figure 5**.

# DISCUSSION

In this study, we reported the influences of 36 h sleep deprivation on working memory, combining behavioral data in two sleep states (baseline and 36 h-TSD) with contemporaneous EEG recordings. The analysis of the results indicated that the changes in the behavioral data in accordance with impaired working memory after 36 h TSD: an increase in the mean reaction time of the cognitive tasks and a decrease in accuracy.

Sleep deprivation impaired the individual's control of attentional resources. Although individuals tried to maintain wakefulness and work performance, including the reaction time and correct rate, during sleep deprivation, the information processing capacity of their working memory was still affected because of the decrease in the speed of processing information (Casement et al., 2006; Wiggins et al., 2018). In this study, the N2 and P3 waves related to working memory were measured to show an increase in latency and a decrease in amplitude after sleep deprivation compared with the baseline readings. Studies have demonstrated that sleep deprivation leads to a continuous decline in attention, and the phenomenon of decreased P3 amplitude indicates that individuals' top-down control of cognition gradually collapses. Sleep deprivation has a more adverse effect on cognitive functions, especially those that depend on mental or cognitions (Kusztor et al., 2019).

The P3 component reflects the deployment of attention resources, and the latency of P3 is widely seen as the time window for stimulus categorization and evaluation. The decrease in the P3 wave amplitude also confirmed that the decision-making in the matching response after TSD had been damaged to a certain extent (Gosselin et al., 2005). Studies have suggested that sleep deprivation can affect the information processing stage of working memory. In this study, the performance indicators also supported the conclusion that the response time to the target stimulus was increased and that the latency of the P3 wave was prolonged (Cote et al., 2008). It was speculated that

**TABLE 2 |** Grand-average peak amplitude of the P2, N2, and P3 components in the correct response condition across multiple electrode sites at baseline and after 36 h-TSD.

| | | Baseline | | | 36 h-TSD | | |
|---|---|---|---|---|---|---|---|
| | | P2 | N2 | P3 | P2 | N2 | P3 |
| F3 | M (SD) | 5.80 (3.89) | −3.61 (4.23) | 7.94 (3.87) | 8.73 (5.55) | −3.50 (5.70) | 6.23 (4.40) |
| Fz | M (SD) | 6.43 (3.82) | −4.14 (4.63) | 8.30 (4.20) | 9.67 (6.12) | −3.99 (6.81) | 6.58 (5.25) |
| F4 | M (SD) | 5.86 (3.94) | −2.82 (4.29) | 8.85 (4.25) | 9.38 (5.88) | −2.96 (5.24) | 6.59 (5.09) |
| C3 | M (SD) | 4.08 (3.08) | −2.37 (4.16) | 8.31 (3.09) | 6.68 (4.76) | −1.67 (5.44) | 6.29 (3.39) |
| Cz | M (SD) | 5.83 (3.63) | −1.89 (5.00) | 9.65 (3.78) | 8.93 (6.09) | −1.34 (6.23) | 7.65 (4.60) |
| C4 | M (SD) | 4.43 (3.31) | −0.45 (3.70) | 9.87 (3.35) | 7.50 (5.00) | −0.35 (4.77) | 7.96 (3.80) |
| P3 | M (SD) | – | −2.12 (6.17) | 8.51 (3.82) | – | −1.39 (5.04) | 8.21 (3.94) |
| Pz | M (SD) | – | −0.82 (4.13) | 9.2 (3.59) | – | 0.02 (4.43) | 8.93 (4.20) |
| P4 | M (SD) | – | −0.36 (4.16) | 7.93 (3.41) | – | 0.32 (5.04) | 8.47 (3.74) |

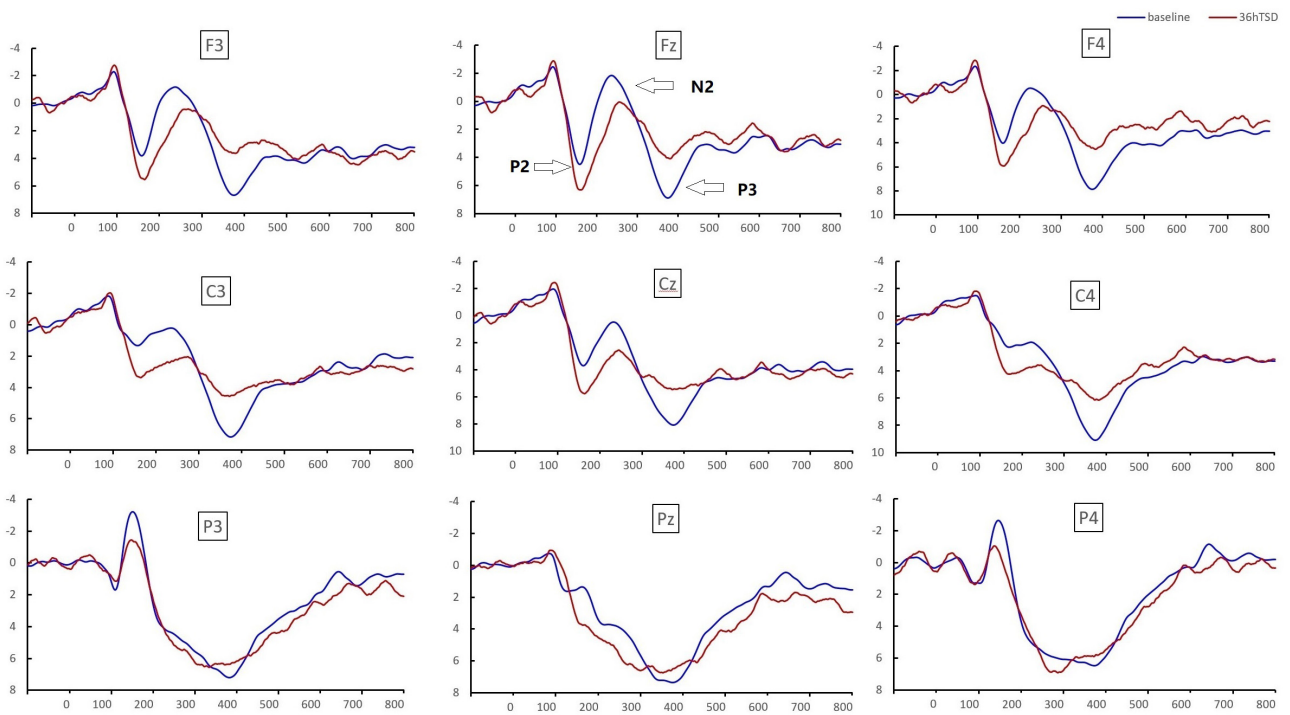


**FIGURE 4 |** ERP amplitude at baseline and 36 h-TSD for the correct response condition for the working memory task. The channels are ordered from left to right and top to bottom as follows: F3, Fz, F4, C3, Cz, C4, P3, Pz, and P4. Compared to the baseline, the latencies of the N2-P3 components were prolonged, and the amplitudes of N2-P3 were decreased after 36 h-TSD.

the effect of sleep deprivation on P3 components might also take place because of the failure to respond to information alter, which is consistent with previous conclusions that the P3 components are related to the updating of working memory content (Donchin and Fabiani, 1991).

Previous studies have considered the N2 component as an electrophysiological index reflecting the ability of the individual to suppress the response (Kreusch et al., 2014). After sleep deprivation, the prolonged latency of the NoGo-N2 component indicates that the individual's ability to suppress the response is impaired (Jin et al., 2015). The decreased amplitude and prolonged latency of the N2 component related to pronunciation working memory after sleep deprivation reveals that sleep deprivation impairs the information processing of pronunciation working memory (Zhang et al., 2019). The N2 component is generally thought to reflect the brain's selective attention and processing of emotional stimuli or signals (Schacht et al., 2008) and is an endogenous component related to an individual's mental state, attention, and degree of attention. In this study, we found that the latency of the N2 component was significantly prolonged, but the amplitude showed only a downward trend. According to previous studies, prolonged N2 latency reflected an increase in response time after sleep restriction (Zhang et al., 2014). However, the finding that N2 amplitude was not

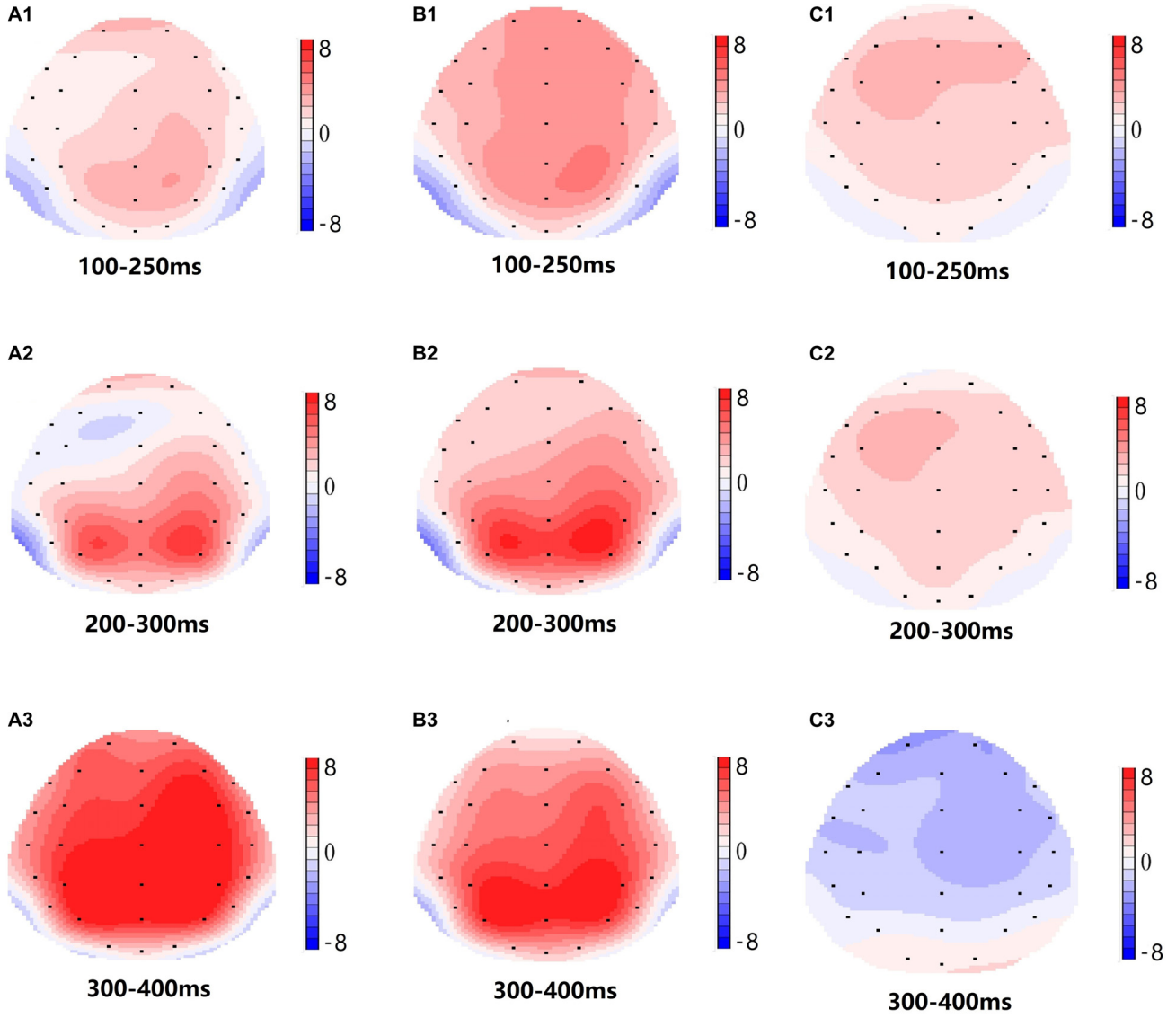


**FIGURE 5 |** Topographic map of the correct response in the working memory task in different sleep conditions **(A1–C3)**. **(A1)** P2, 100–250 ms, at baseline. **(A2)** N2, 200–300 ms, at baseline. **(A3)** P3, 300–400 ms, at baseline. **(B1)** P2, 100–250 ms, at 36 h-TSD. **(B2)** N2, 200–300 ms, for 36 h-TSD. **(B3)** P3, 300–400 ms, for 36 h-TSD. **(C1)** P2, 100–250 ms, 36 h-TSD with baseline subtracted. **(C2)** N2, 200–300 ms, 36 h-TSD with baseline subtracted. **(C3)** P3, 300–400 ms, 36 h-TSD with baseline subtracted.

significantly altered may have been due to cerebral compensatory responses (Drummond and Brown, 2001). In the case of limited cognitive resources, there was a compensation mechanism to restore impaired cognitive function (Jin et al., 2015).

According to the scalp topography, the changes in the N2-P3 components related to sleep deprivation are more obvious in the frontal area. Frontoparietal control (FPC) plays an important role in cognitive control. Studies have shown that FPC can bypass top-down cognitive control, enabling individuals to focus on information related to the target while suppressing information that is not related to the target (Smallwood et al., 2011; Wen et al., 2013). FPC is important for information retention and information processing in working memory, and the degree of activation of FPC after sleep deprivation was reduced compared to a normal sleep group (Ma et al., 2014). Although the EEG results did not reflect the changes in specific brain regions in detail, it intuitively reflected the effect of TSD on the retention and processing of working memory information.

Although the exact cognitive process that the P2 component underlies is still widely debated, as a broad definition, the P2 component reflects the process of attention and visual processing and is generally considered to be related to selective attention and working memory, reflecting the early judgment of the perceptual process (Saito et al., 2001). In this study, we found a significant increase in the P2 wave amplitude after sleep

**TABLE 3 |** Grand-average peak latency of the P2, N2, and P3 components in the correct response condition across multiple electrode sites at baseline and after 36 h-TSD.

| | | Baseline | | | 36 h-TSD | | |
|---|---|---|---|---|---|---|---|
| | | P2 | N2 | P3 | P2 | N2 | P3 |
| F3 | M (SD) | 164.96 (22.60) | 245.05 (27.17) | 376.55 (20.25) | 173.65 (23.80) | 262.01 (40.37) | 379.11 (25.86) |
| Fz | M (SD) | 164.71 (22.48) | 243.36 (25.49) | 374.75 (19.90) | 175.11 (22.87) | 260.72 (31.55) | 382.70 (29.48) |
| F4 | M (SD) | 165.87 (23.54) | 239.46 (27.33) | 373.20 (19.92) | 174.66 (24.87) | 261.86 (39.24) | 380.26 (25.93) |
| C3 | M (SD) | 167.69 (26.33) | 234.64 (28.98) | 370.71 (19.19) | 179.20 (27.57) | 238.39 (40.16) | 363.80 (32.50) |
| Cz | M (SD) | 166.58 (20.32) | 236.33 (31.09) | 362.17 (24.53) | 176.27 (25.83) | 238.04 (40.83) | 366.19 (42.65) |
| C4 | M (SD) | 174.63 (24.57) | 230.75 (33.37) | 368.18 (18.65) | 189.29 (26.59) | 242.18 (44.43) | 365.59 (35.13) |
| P3 | M (SD) | – | 218.71 (54.68) | 354.11 (29.25) | – | 213.39 (41.47) | 357.09 (44.99) |
| Pz | M (SD) | – | 213.57 (37.03) | 350.28 (34.14) | – | 229.17 (46.15) | 341.13 (41.06) |
| P4 | M (SD) | – | 221.30 (54.50) | 326.72 (38.87) | – | 227.36 (50.51) | 324.11 (48.03) |

deprivation. Studies have reported that P2 waves which might be a part of the early cognitive matching system for message processing and may compare sensory inputs to stored memory (Freunberger et al., 2007) are sensitive to alterations in mission attention and working memory demands (Smith et al., 2002). Functional compensation is one of the unique functions of the human brain and an important factor for maintaining cognitive function. Excessive activation of the dorsolateral prefrontal cortex (DLPFC) after sleep deprivation indicates that, as brain resources decrease, the DLPFC appears to have a compensatory function (Drummond et al., 2004; Choo et al., 2005). Therefore, we speculate that the significant increase in P2 amplitude observed in this study may be due to functional compensation in which individuals appear to maintain normal cognitive function after sleep deprivation. Although a large number of studies have used ERP technology to explore the effect of sleep deprivation on cognitive functions, early components such as N1 and P2 have not been systematically studied, and the results are inconsistent (Evans and Federmeier, 2007; Wiggins et al., 2018; Zhang et al., 2019). There are few researches explore the change of P2 component during sleep deprivation (Mograss et al., 2009). Therefore, the effects of sleep deprivation on early components of ERP, such as P2, still need to be further studied and explored.

In this experiment, we used the 2-back model to design pronunciation, spatial, and object working memory tasks and examined the impairment of working memory after 36 h of TSD. Compared with previous studies that focused only on the effects of sleep deprivation on a specific type of information, such as pronunciation working memory, or specific cognitive function, such as response inhibition, we have considered the contents of the working memory model and comprehensively analyzed the effects of sleep deprivation on working memory.

However, the study has some limitations. First, we only used the 2-back task and failed to compare the performance of the participants in working memory tasks of different difficulties. Therefore, there are limitations in explaining and inferring changes in workload. Second, only male volunteers were used in the study, and the conclusions need to be assessed when extending them to female volunteers. Due to the limited number of participants, we found only that the amplitude of the N2 wave had a downward trend and that the P3 wave latency had a prolonged trend. Stable results might be obtained after increasing the number of participants. Third, combining our procedure with fMRI for working memory may facilitate further interpretation of the results. Previous studies have shown that circadian biorhythms affect behavioral performance, and there are individual differences (Montplaisir, 1981; Lavie, 2001). We did not record the EEG data at the same time point in this experimental, so the influence of circadian biorhythms on the test results cannot be completely ruled out.

This research showed that working memory ability was impaired after TSD and that this damage was not associated with the stimulus content of working memory. The lack of sleep reduced the quality of the information stored in memory, which might occur with the degenerative process of attention (Ratcliff and Van Dongen, 2018). This study provides electrophysiology evidence for understanding the mechanism under the impaired working memory after sleep deprivation. It is necessary to pay attention to the adverse effects of working memory impairment caused by sleep deprivation and to explore effective interventions for such damage.

## DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Fourth Military Medical University Beihang University. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

YS designed the experiments. ZP produced the results and wrote the manuscript. CD and LZ analyzed and interpreted

the data. JT and YS performed the experiments, acquainted the data, and the guarantors of this study. YB, LZ, and JT contributed to participating in data collection and reviewing the literature. All authors listed have read and approved the final manuscript.

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# Functional Connectivity Abnormalities of Brain Regions With Structural Deficits in Primary Insomnia Patients

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**Objectives:** The present study examined the abnormal resting state functional connections (RSFCs) in structural deficit brain regions of primary insomnia (PI) patients.

**Methods:** Thirty-three PI patients and 38 well-matched healthy controls participated in our study. We used voxel-based morphometry and RSFC to study functional connectivity abnormalities of brain regions with structural deficits in PI patients.

**Results:** PI patients showed decreased gray matter (GM) volume in the left dorsolateral prefrontal cortex, left orbitofrontal cortex (OFC), bilateral middle frontal gyrus (MFC), right inferior frontal gyrus (IFG), and left inferior temporal gyrus. Gray matter volume in the right MFC negatively correlated with Self-Rating Scale of Sleep (SRSS) scores, and GM volume in the right IFG negatively correlated with SRSS and Insomnia Severity Index (ISI) scores. Therefore, the right MFC and right IFG were selected as regions of interest for RSFC analysis. PI patients had weakened RSFC between the right inferior parietal gyrus (IPC) and the right MFC compared to the healthy controls and between the left OFC and right IFG. The RSFC between the right MFC and right IPC negatively correlated with SRSS scores. The RSFC between the right IFG and left OFC negatively correlated with SRSS, ISI, SAS, and SDS scores.

**Conclusions:** The present study found structural changes in the right MFC and right IFG accompanied by RSFC changes. This finding may provide novel insights into the neural mechanisms of PI *via* combining structural and functional modality information.

**Keywords: primary insomnia, voxel-based morphometry (VBM), resting state functional connectivity, middle frontal gyrus, inferior frontal gyrus**

## INTRODUCTION

As one of the most common health problems in people and in clinical practice (Lichstein et al., 2003), primary insomnia (PI) generally manifests as difficulties in the onset and maintenance of sleep (Morin et al., 2011). Primary insomnia is present in 6–10% of the total population, especially women and older adults (Xu et al., 2011; Bélanger et al., 2012; Buysse, 2013), which may influence

their quality of life and produce physical impairment, mental disorders, and increased medical care costs (Buysse, 2013). Over the past few decades, many neuroimaging studies investigated the neural mechanism of PI and found extensive structural and functional changes in several brain regions. Unfortunately, the neurobiological mechanism of PI is not clear.

Brain imaging technology is a useful tool for studying the neural mechanism of PI. Many structural neuroimaging studies used voxel-based morphometry (VBM) analysis to reveal PI-related brain tissue injury (Yeon et al., 2013), and multiple brain regions showed abnormal gray matter (GM) volume. Primary insomnia patients revealed decreased GM volume in the hippocampus, precuneus, middle temporal gyrus, left orbitofrontal cortex (OFC), the bilateral dorsolateral prefrontal cortex (DLPFC), left middle frontal gyrus (MFC), and the bilateral inferior frontal gyrus (IFG; Dieter et al., 2007; Altena et al., 2010; Stoffers et al., 2012; Joo et al., 2013; Li et al., 2018) compared to healthy controls. Functional neuroimaging methods were also used to study brain functional abnormality in PI patients. Resting state functional connectivity (RSFC) is primarily used to examine spontaneous neuronal activity in the human brain, which may provide new insights into the neurological mechanism of abnormal behavior in PI patients (Dijk et al., 2010; Friston, 2011). Seed-based connectivity analysis revealed decreased RSFC between the parietal lobe and the frontal lobe (Li et al., 2014) and between the left OFC and left caudate (Diederick et al., 2014). Our previous study found reduced RSFC between the thalamus and hippocampus, anterior cingulate cortex (ACC), OFC, caudate, and putamen (Min et al., 2018). Most previous studies focused on the difference in RSFC between PI patients and healthy subjects, but few studies investigated the RSFC in brain structural defect regions of PI patients in the whole brain.

The structural changes may be accompanied by impairment of brain function. Multimodal technical methods that combine structure and function may provide more useful information for clinical diagnosis. The combination of VBM and FC was used to study schizophrenia (Zhang et al., 2015), Parkinson's disease (Canu et al., 2015), and temporal lobe epilepsy (Doucet et al., 2016). To our knowledge, no previous study has investigated the neurobiological mechanisms of PI using a combination of VBM and FC analysis. The present study detected common and distinct brain structural and functional alterations between PI patients and healthy controls based on brain structural and functional images. The current study enrolled relatively homogeneous subjects to investigate structural abnormalities between PI patients and a control group. Changes in brain RSFC with structural deficits were assessed. We hypothesized that brain regions with structural deficits in PI patients would show abnormal RSFC, and these changes would be associated with sleep behaviors. The combination of structural and functional methods may provide new perspectives on the neural mechanism of PI.

# MATERIALS AND METHODS

The Medical Ethics Committee of the First Affiliated Hospital of Baotou Medical College, Inner Mongolia University of Science and Technology approved all procedures. Before the experimental study, a researcher informed the participants of the possible risks and discomfort of participating in the study. All of the participants volunteered to participate in the study and provided written informed consent.

## Participants

Subjects were recruited from the First Affiliated Hospital of Baotou Medical College, Inner Mongolia University of Science and Technology, Baotou, China. Thirty-three adults with PI (10 males, 23 females; mean ± standard deviation age = 42.27 ± 9.27 years) and 38 age-, gender-, education-matched healthy control subjects (15 males, 23 females; mean ± standard deviation age = 43.72 ± 10.10 years) participated in the present study. The following inclusion criteria for PI patients were used: (1) age 18–68 years; (2) right-handed, Han nationality; (3) meeting the criteria for sleep disturbance in the DSM-V and lasting for more than 3 months; (4) reporting difficulty in initiating and maintaining sleep or early awakening for at least 1 month; and (5) PSQI total score $\geqq$ 8. The following exclusion criteria were used: (1) intellectual disability, mental disorders (such as epilepsy) or chronic pain diseases; (2) patients with serious primary diseases, such as cancer, diseases of the heart, liver, lung, kidney, hematopoietic system, and other diseases; (3) pregnant or lactating women, allergic constitution and allergic to a variety of drugs; (4) MRI examination showed organic occupying, bright signal or other structural abnormalities in the brain; (5) medication or substance abuse, such as alcohol, nicotine, or other drugs; and (6) MRI contraindications (cardiac pacemaker, insulin pump, artificial heart valve, and others with metal in the body, or claustrophobia). The following inclusion criteria were used for healthy controls: (1) good sleep quality, no difficulty in starting or maintaining sleep; and (2) PSQI total score <5. The exclusion criteria were the same as the PI patients. All subjects completed a number of self-questionnaires, such as the Pittsburgh Sleep Quality Index (PSQI), Insomnia Severity Index (ISI), Self-Rating Scale of Sleep (SRSS), Self-Rating Anxiety (SAS), and Self-Rating Depression Scale (SDS). The study protocol was performed in compliance with the Declaration of Helsinki and approved by the research Ethical Committee of First Affiliated Hospital of Baotou Medical College, Inner Mongolia University of Science and Technology.

## Data Acquisition

Magnetic resonance imaging (MRI) was performed using a 3T Philips scanner (Achieva; Philips Medical Systems, Best, Netherlands) at the First Affiliated Hospital of Baotou Medical College, Inner Mongolia University of Science and Technology, Baotou, China. The head of the subject was fixed in a foam pad and kept in a comfortable position before the scan. Subjects wore earplugs to reduce the loud noise from the machine. A high-resolution T1 structural

image was obtained using a magnetization-prepared rapid acquisition gradient echo (MPRAGE) pulse sequence and the following scanning parameters: repetition time/echo time (TR/TE) = 8.5/3.4 ms; matrix = 240 × 240; slices = 140; and field of view (FOV) = 240 mm × 240 mm; and voxel size = 1 mm × 1 mm × 1 mm. Resting state functional images were scanned using an echo-planar imaging (EPI) sequence (slices = 32; slice thickness = 5 mm; TR/TE = 2000/30 ms; flip angle (FA) = 90°; FOV = 240 mm × 240 mm; data matrix = 64 × 64; total volumes = 185). Subjects remained awake with their eyes closed during the entire scan and were told not to think about anything. At the end of scanning, the participants were asked if they were awake during the functional data collection. Two radiologists examined the images of all participants to exclude any aspect of clinical pathological changes in any subject.

## Data Analysis

### VBM Analysis

All T1 data preprocessing were performed using the Statistical Parametric Mapping 8 software (SPM8; Wellcome Department of Cognitive Neurology) and VBM8 toolbox (University of Jena, Department of Psychiatry) run in Matlab2011a. First, the original T1 data were registered to the Montreal Neurological Institute (MNI) template to match the brain structure images of different subjects in the same space. Subsequently, the structure images were segmented into GM, white matter (WM), and cerebrospinal fluid (CSF) using an integrated one-pass procedure (the unified segmentation). The segmented GM images were modulated to correct the volume change after standardization. Finally, GM images were smoothed with an 8-mm full-width at half-maximum Gaussian kernel to reduce noise and to improve the signal-to-noise ratio. To compare changes in GM volume between PI patients and healthy controls, a two-sample t-*t*est was performed in SPM8 [familywise error (FWE) correction, $p < 0.05$].

### RSFC Analysis

Resting state data preprocessing was performed using Analysis of Functional NeuroImages (AFNI)[1] and FMRIB Software Library (FSL)[2] as described in our previous studies (Bi et al., 2016; Yuan et al., 2016a,b; Min et al., 2018). Functional data preprocessing was divided into core image processing and denoising. In general, the preprocessing procedure of core images included the following steps: (Lichstein et al., 2003) removal of the first 5 volumes of the functional image; (Morin et al., 2011) slice timing correction; (Bélanger et al., 2012) head motion correction (3° rotations and 3-mm displacements); (Xu et al., 2011) obliquity transformation to the structural image; (Buysse, 2013) affine co-registration to the skull-stripped structural image; (Yeon et al., 2013) spatial normalization to the MNI152 template; (Altena et al., 2010) spatial smoothing (4 mm full-width at half-maximum Gaussian kernel); and (Li et al., 2018) intensity normalization to a whole-brain median of 1000. Previous studies found that nuisance regression and bandpass filtering alone were generally inadequate to control head motion-induced noise (Power et al., 2012; Patel et al., 2014). Therefore, wavelet denoising is used in the study of RSFC (Patel et al., 2014). The denoising steps included: time series despiking (wavelet domain); nuisance signal regression, including the six motion parameters, their first-order temporal derivatives, WM and CSF signal (14-parameter regression); and a temporal Fourier filter (0.009–0.10HZ).

Brain regions that showed altered GM volume between PI patients and healthy controls were defined as the regions of interests (ROIs). Using these ROIs as seed points, the average time series was extracted as the reference time series, and a figure relevant to functional connectivity was obtained between the average fMRI time series of each ROI and all voxels in the brain using the Resting State fMRI Data Analysis Toolkit (REST1.8)[3] software (Xiao-Wei et al., 2011). The R value maps were transformed into approximate Gaussian distributions using a Fisher's *Z* transformation. Finally, two-sample *t*-test for *Z* values was performed to observe these brain regions related to the seed points in SPM8 ($p < 0.05$, FWE corrected).

## Correlation Analysis

Pearson correlation analysis was used to examine the relationship between GM volume and clinical variables (SRSS, PSQI, ISI, SAS, and SDS). Brain regions with abnormal GM volumes were deemed ROIs. The average GM volume of these ROIs were extracted using REST1.8. The correlation analysis compared the mean GM volume and the scale scores using IBM SPSS statistics (version 20.0, SPSS Inc, Chicago, IL, United States). Similarly, REST1.8 software was used to extract the functional connectivity strength and the *Z* value of each ROI from the brain regions with abnormal functional connectivity in the two groups. Pearson analysis was used to investigate the relationships between the clinical measures and *Z* values in PI patients. $P < 0.05$ was considered statistically significant.

## Statistical Analyses

Non-imaging data statistical analyses were performed using SPSS version 20.0. Chi-squared test was used to compare the gender difference in the two groups. Two-tailed two-sample *t*-tests were used for SAS, SDS SRSS, PSQI, and age. The significance level was set at $p < 0.05$.

# RESULTS

## Demographic and Clinical Characteristics

The detailed demographic characteristics in the current study are given in **Table 1**. There were no significant differences in gender or age ($p > 0.05$) between the PI and control groups. However, the PI patients showed higher SRSS, PSQI, SDS, and SAS scores than controls, as expected (**Table 1**).

[1] http://afni.nimh.nih.gov/

[2] http://fsl.fmrib.ox.ac.uk/fsl/

[3] http://www.restfmri.net/forum/REST

**TABLE 1 |** Demographic characteristics of primary insomnia (PI) patients and healthy controls (HC) in the present study.

| | PI (*N* = 33) | HC (*N* = 38) | *P* value |
|---|---|---|---|
| Male/female | 10/23 | 15/23 | 0.651 |
| Age (years) | 42.27 ± 9.27 | 43.72 ± 10.10 | 0.768 |
| SRSS | 34.94 ± 6.92 | 16.33 ± 2.25 | <0.001 |
| PSQI | 13.54 ± 3.54 | 3.86 ± 2.21 | <0.001 |
| SAS | 53.75 ± 10.09 | 24.31 ± 3.49 | <0.001 |
| SDS | 47.04 ± 9.60 | 16.00 ± 9.77 | <0.001 |
| ISI | 17.63 ± 6.30 | – | – |

*Chi-squared test was used for gender comparisons; two-tailed two-sample t-tests were used for SRSS, PSQI, SAS, SDS and age comparisons. The significance level was set as $p < 0.05$. Values are expressed as the means ± standard deviations. SRSS, Self-Rating Scale of Sleep; PSQI, Pittsburgh Sleep Quality Index; SAS, Self-Rating Anxiety; SDS, Self-Rating Depression Scale; ISI: Insomnia Severity Index.*

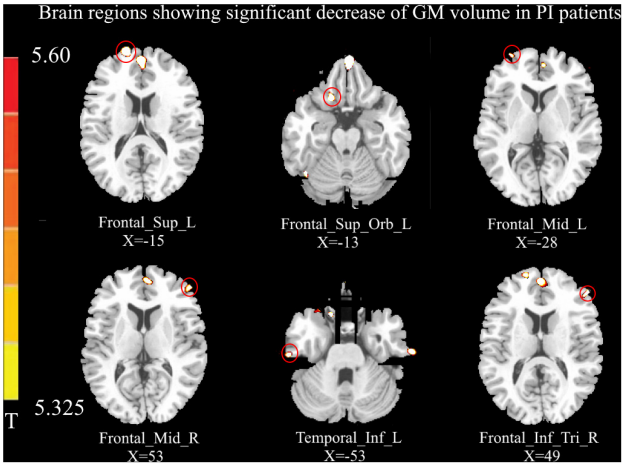


**FIGURE 1 |** Compared with healthy controls ($p < 0.05$, FEW corrected), the volume of gray matter (GM) in the left dorsolateral prefrontal cortex (DLPFC), left orbital prefrontal cortex (OFC), bilateral middle frontal gyrus (MFC), left inferior temporal gyrus, and right inferior frontal gyrus (IFG) was decreased in primary insomnia (PI) patients. No increased GM volume was observed in the brain regions of PI patients in this study.

## VBM Results

Relative to healthy controls, the volume of GM in several brain regions was decreased in PI patients, i.e., the left DLPFC, left OFC, bilateral MFC, right IFG, and left inferior temporal gyrus ($p < 0.05$, FWE corrected; **Figure 1**). However, there was no increase in GM volume in the brain region of PI patients in this study. The correlation analysis of GM volume and behavioral data are shown in **Figure 2**.

## Functional Connectivity Results

Using the right MFC and right IFG as RIOs, two-sample t-tests revealed significantly lower RSFC between the right MFC and right inferior parietal gyrus (IPC) in the PI group compared to the control group. RSFC between the right IFG and left OFC was significantly weakened in the PI group (**Figure 3A**, $p < 0.05$, FWE corrected).

## Correlation Analysis Results

The GM volume of the right MFC negatively correlated with SRSS score in PI patients (**Figure 2A**, $p = 0.003$). The GM volume of the right IFG negatively correlated with the SRSS score (**Figure 2B**, $p < 0.001$) and ISI score (**Figure 2C**, $p = 0.006$). The abnormalities in functional connectivity were associated with the scores of the sleep self-questionnaires. The RSFC between the right MFC and right IPC negatively correlated with the SRSS score (**Figure 3B**, $p = 0.035$). The RSFC between the right IFG and left OFC negatively correlated with the SRSS score (**Figure 3B**, $p = 0.002$), ISI score (**Figure 3B**, $p = 0.036$), SAS score (**Figure 3B**, $p = 0.027$) and SDS score (**Figure 3B**, $p = 0.038$).

# DISCUSSION

The present study combined the VBM and RSFC methods to investigate structural and functional changes in PI patients. Compared to healthy controls, the volume of GM in several brain regions was decreased in PI patients, i.e., the left DLPFC, left OFC, bilateral MFC, right IFG, and left inferior temporal gyrus (**Figure 1**). The GM volume in the right MFC negatively correlated with the SRSS score (**Figure 2A**), and GM volume in the right IFG negatively correlated with the SRSS score (**Figure 2B**) and ISI score (**Figure 2C**). PI patients showed decreased RSFC between the right IPC and right MFC, and between the left OFC and right IFG (**Figure 3A**). The RSFC between the right MFC and right IPC negatively correlated with SRSS scores, and the RSFC between the right IFG and left OFC negatively correlated with ISI, SRSS, SAS, and SDS scores (**Figure 3B**).

Previous studies consistently confirmed the structural deficits in the prefrontal cortex (PFC) in PI subjects (Altena et al., 2010; Stoffers et al., 2012; Joo et al., 2013; Li et al., 2018). We found a decrease in the volume of GM in the DLPFC, including the middle and inferior prefrontal regions, in the PI patients. Consistent with previous findings (Joo et al., 2013; Li et al., 2018), PI patients showed a decreased GM volume in the MFC and IFG. As a key node of the default mode network (DMN), the MFC is related to the onset and maintenance of sleep (Koenigs et al., 2010). The decreased GM volume and lower regional homogeneity of MFC was associated with PI in previous studies (Joo et al., 2013; Dai et al., 2014). Recent studies revealed that the IFG is a main cortical hub in the brain network affected by PI (Yan et al., 2018). Stoffers et al. found that subjects with lower GM density in the left IFG reported more early morning awakening in an independent sample of people not diagnosed with insomnia, which may be translated into complaints of PI (Stoffers et al., 2012). Jiang et al. examined low frequency fluctuations (ALFFs) and observed lower ALFF values in the left IFG, and the PI duration negatively correlated with the ALFF value of the left IFG (Jiang et al., 2016). Recent studies observed a decrease in the DC value of the left IFG, and the DC value of PI patients positively correlated with PSQI (Yan et al., 2018). Therefore, we speculated that the IFG was a vulnerable area in the pathological process of PI. One recent study showed that sleep was susceptible to

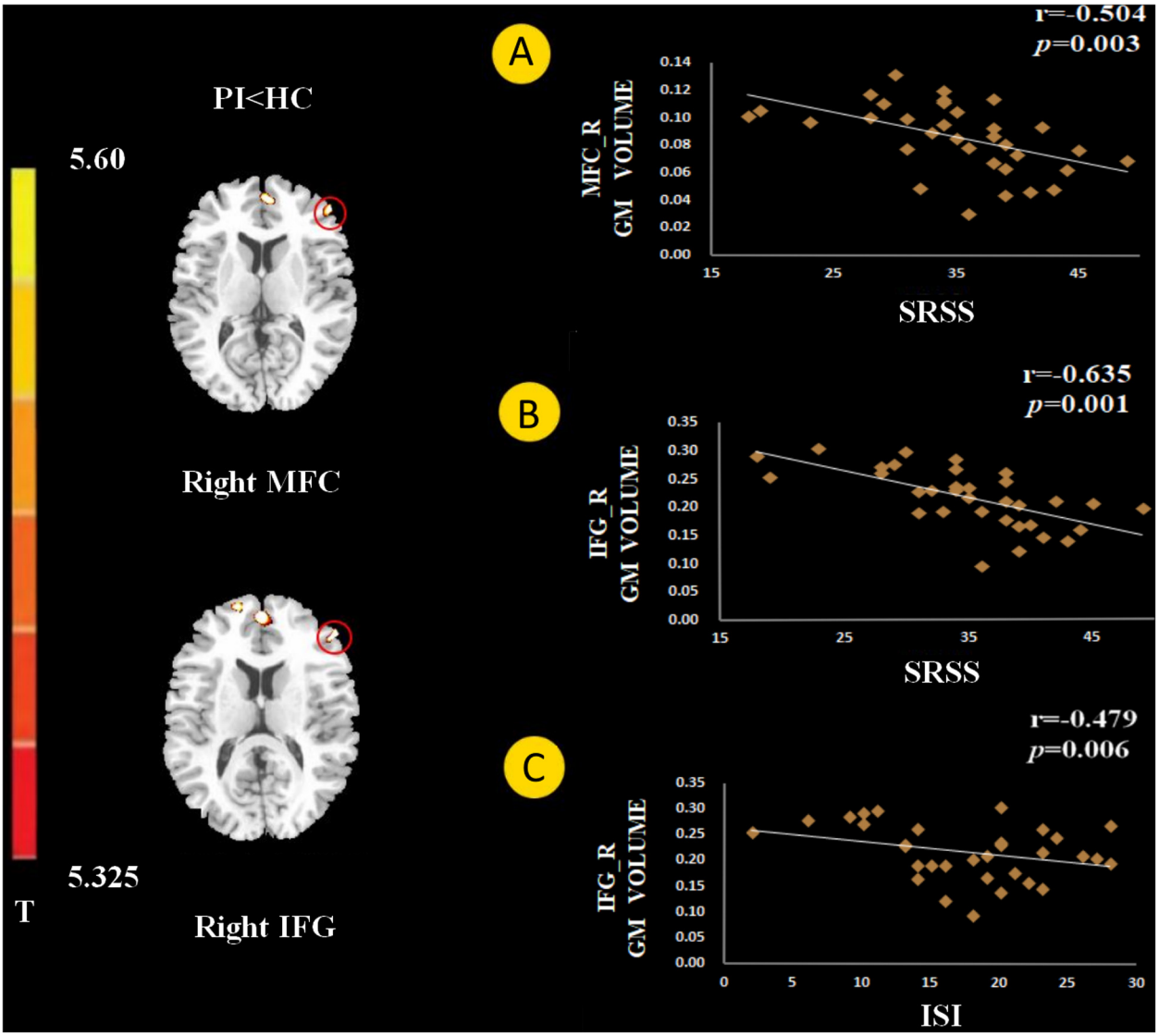


**FIGURE 2 |** The gray matter (GM) volume in the right middle frontal gyrus (MFC) negatively correlated with Self-Rating Scale of Sleep (SRSS) score **(A)**. The GM volume of the right inferior frontal gyrus (IFG) negatively correlated with SRSS score **(B)**, and Insomnia Severity Index (ISI) score **(C)**.

GM deficits in the PFC, and abnormality of the DLPFC may be related to PI complaints, such as early rising and difficulty falling asleep (Li et al., 2018). The PFC is associated with higher-order cognitive function, including decision-making and executive ability (Gläscher et al., 2012; McNamee et al., 2013; Squire et al., 2013). A study of a vigilance task that was associated with decision-making found that PI patients responded more slowly than the control group (Altena et al., 2008). Decision-making in previous studies was very sensitive to sleep deprivation (Vinod et al., 2007). Therefore, insomnia may be related to the abnormal volume of GM in the PFC, which leads to a decline of decision-making ability.

The SRSS of patients with PI negatively correlated with GM volumes in the MFC, and the IFG GM volumes negatively correlated with ISI (**Figure 2**). These preliminary results showed a significant negative correlation, which may support a relationship between the PFC and sleep quality.

Our study investigated changes in brain RSFC with structural deficits. RSFC may reveal interregional functional changes *via* the calculating of the temporal correlation between the internal fluctuations observed in spatially different brain regions in resting states (Fox and Raichle, 2007; Biswal et al., 2010). Compared with the healthy controls, PI patients showed weakened RSFC between the right MFC and right IPC and between the right IFG and left OFC (**Figure 3A**). The IPC is a large and heterogeneous region and an important node of the DMN. Previous sleep deprivation studies found that spontaneous activity in the IPC region and its connection patterns with other DMN subregions were abnormal after 72 h of sleep deprivation (Dai et al., 2015). These studies suggest

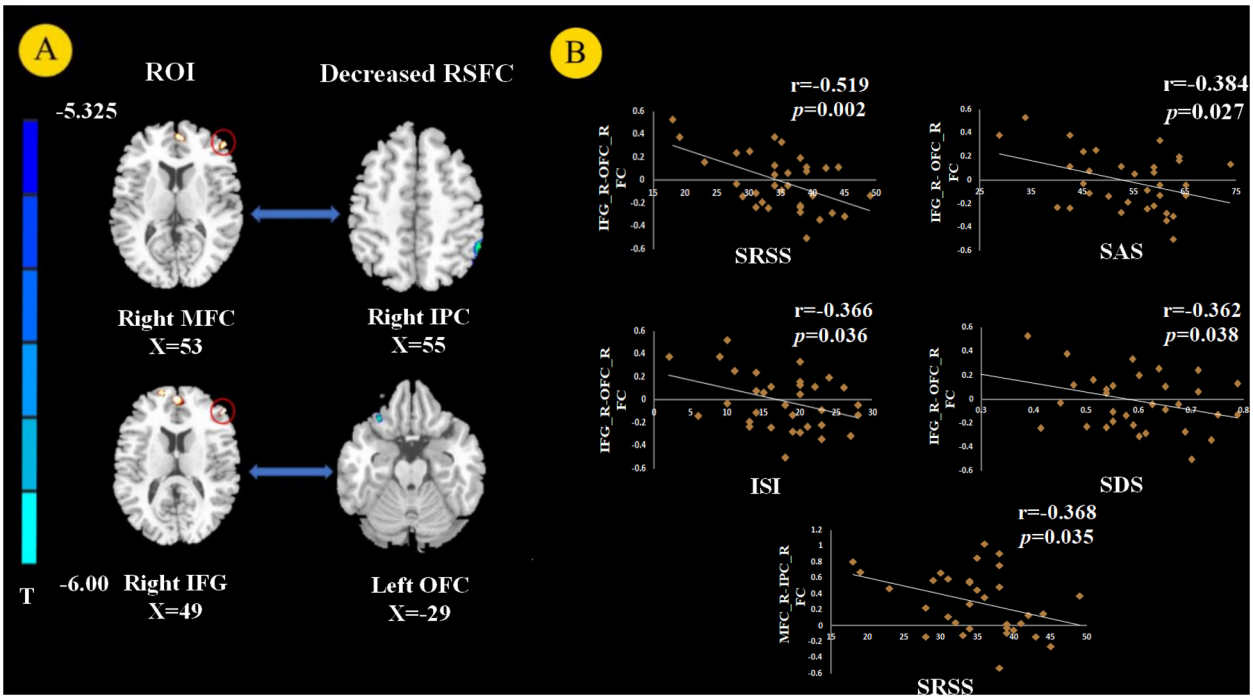


**FIGURE 3 |** Resting state functional connectivity (RSFC) analysis ($p < 0.05$, FEW corrected). We chose the right MFC and right IFG as the regions of interest (ROIs) and found decreased RSFC between the right MFC and right inferior parietal gyrus (IPC) and between the right IFG and left orbital prefrontal cortex (OFC) in PI patients **(A)**. The RSFC between the right MFC and right IPC negatively correlated with SRSS score. The RSFC showed negative correlation with the SRSS, ISI, SAS, and SDS scores between the right IFG and left OFC in PI patients **(B)**.

that abnormal spontaneous activity and connectivity in the IPC are associated with sleep disorders. Horovitz et al. (2009) found that the RSFC between IPC and MFC decreased after deep sleep or partial sleep deprivation (Sämann et al., 2010). Li et al. (2014) used rs-fMRI and seed-based connectivity analysis and found that the connection between the bilateral parietal lobe and right frontal lobe was weak in PI patients. They suggested that the reduced interaction between the parietal and frontal lobes were responsible for spatial and verbal working memory deficits caused by insomnia (Walter et al., 2003). The activation of frontal and parietal lobes was lower in PI patients in a spatial memory task (Li et al., 2016). Many studies confirmed that PI patients had defects in attention processes and working memory tasks (Fortier-Brochu et al., 2012; Drummond et al., 2013). Previous research found that the frontoparietal network (FPN) was closely related to attention-keeping and working memory (Corbetta and Shulman, 2002; Seeley et al., 2007). Therefore, connection dysfunction in the FPN may be related to the pathophysiology of cognitive impairment in PI. We also observed that the significant reduction in FPN RSFC in PI patients was related to poor sleep quality, as assessed by SRSS, which may further explain the underlying neurobiological mechanism of FPN RSFC and insomnia.

We discovered that PI patients showed decreased RSFC between the right IFG and left OFC compared to good sleepers. Notably, the RSFC between the right IFG and left OFC negatively correlated with SRSS and ISI scores. The relationship between OFC and sleep quality was most frequently assessed using MRI in the sleep literature (Sexton et al., 2014). A VBM study demonstrated that PI patients had significantly reduced GM density in the left OFC (Altena et al., 2010). More importantly, the decreased GM density of the left OFC may represent a pre-existing vulnerability to sleep complaints (Stoffers et al., 2012). FMRI studies found a weakened amplitude of low-frequency fluctuations (ALFF) in the OFC after sleep deprivation (Lei et al., 2015). The OFC involves the ability to make decisions and solve problems, and it is related to sleep deprivation (Venkatraman et al., 2007). The OFC region monitors the intensity of heat stimulation in the surrounding environment (Rolls et al., 2008). Therefore, decreased activation of the OFC of PI patients may be related to their weakened understanding of optimal sleep comfort environment temperature. The decreased right IFG – left OFC RSFC correlated with SAS and SDS scores, which indicates a possible link between the RSFC of IFG-OFC with depression and anxiety. The OFC is a core brain area of the emotional arousal and motivation brain network (Olausson et al., 2007). Abnormalities in the OFC in emotion disorders were demonstrated using functional neuroimaging (Rolls, 2019). Previous research found that the lateral OFC area was associated with depression because it responded to not receiving the expected rewards, which is a typical cause of sadness and depression (Rolls, 2000, 2016, 2019). Notably, a negative correlation between the SDS and RSFC between the right IFG and left OFC was also found in healthy controls. The PFC plays an important role in emotional

processing (Etkin et al., 2011). Our current study provides evidence that impaired connectivity of the PFC, especially the MFC and IFG, may be related to poor sleep quality in insomnia. However, connectivity in the IFG may be associated with depression in normal people.

However, there are some limitations in the current study. First, this cross-sectional study cannot lead to causal conclusions between PI and structural and functional abnormalities of the brain. Longitudinal studies may be performed in the future to determine whether these changes are a cause of insomnia or a consequence of long-term sleep deprivation. The number of subjects in this paper may be too small.

## CONCLUSION

In summary, our study used a multimodal approach that combined VBM analysis and resting-state functional connection analysis to investigate structural and functional abnormalities in the brains of PI patients. We found structural changes in the right MFC and right IFG accompanied by RSFC changes. These results indicate that VBM and RSFC effectively evaluated changes in PI-related brain structure and functional connectivity. We hope our research provides some theoretical basis for the study of PI neuropathology and ideas for clinical application.

## DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Research Ethical Committee of First Affiliated Hospital of Baotou Medical College, Inner Mongolia University of Science and Technology approved the trial. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

DX and FD: study design, data analysis, and manuscript writing. XW, CL, and HQ: data collection. TX: data analysis. KY, BL, and YH: manuscript revision. DY: study design. All authors contributed to the article and approved the submitted version.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2020.00566/full#supplementary-material

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# Total Sleep Deprivation Impairs Lateralization of Spatial Working Memory in Young Men

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Total sleep deprivation (TSD) negatively affects cognitive function. Previous research has focused on individual variation in cognitive function following TSD, but we know less about how TSD influences the lateralization of spatial working memory. This study used event-related-potential techniques to explore asymmetry in spatial-working-memory impairment. Fourteen healthy male participants performed a two-back task with electroencephalogram (EEG) recordings conducted at baseline and after 36 h of TSD. We selected 12 EEG points corresponding to left and right sides of the brain and then observed changes in N2 and P3 components related to spatial working memory. Before TSD, P3 amplitude differed significantly between the left and right sides of the brain. This difference disappeared after TSD. Compared with baseline, P3 amplitude decreased for a duration as extended as the prolonged latency of N2 components. After 36 h of TSD, P3 amplitude decreased more in the right hemisphere than the left. We therefore conclude that TSD negatively affected spatial working memory, possibly through removing the right hemisphere advantage.

**Keywords: sleep deprivation, spatial working memory, event-related potentials, lateralization, n-back**

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## INTRODUCTION

Sleep quality and time spent sleeping have decreased gradually with increasing social pressure and acceleration of life pace in recent years; however, sleep loss negatively affects productivity and sleep disturbance can lead to various diseases. Total sleep deprivation (TSD) damages brain function, leading to deficits in alertness, attention, and working memory (Durmer and Dinges, 2005; Lim and Dinges, 2010; Basner et al., 2013). Post-TSD decreases in short-term memory are associated with abnormal functional connections between the hippocampus and the cerebral cortex (Li et al., 2016). After TSD, participant responses to working memory tasks were significantly slower (Gujar et al., 2010). Furthermore, positive activation of the medial parietal cortex was significantly reduced, negative activation of the anterior medial frontal lobe and posterior cingulate decreased, while activation of left dorsal lateral prefrontal and bilateral thalamus increased (Gujar et al., 2010). Total sleep deprivation also causes declines in learning and memory (Kusztor et al., 2019).

Working memory is a system that temporarily stores and maintains a limited amount of information to complete a specific task. Working memory consists of the central executive,

visuospatial sketchpad, phonological loop, and episodic buffer (Baddeley, 1992). The visuospatial sketchpad comprises two independent parts: visual working memory and spatial working memory. Spatial working memory involves retaining the locations where objects have appeared (Huntley et al., 2011). A topic of interest in cognitive neuroscience is therefore the activation of specific brain regions during these various working-memory processes.

Functional asymmetry is a very important feature of human brain function, with one cerebral hemisphere controlling particular tasks more than the other. This asymmetry is particularly evident in language, visual spatial processing, and emotional expression. Thus, clarifying the nature of this asymmetry is critical for understanding functional neural-processing organization (Ma and Han, 2012). Available neuropsychological research indicates that the right hemisphere of the brain has the advantage in attention to spatial positioning. The right hemisphere is effective in coding, emotional expression, processing and synthesis of overall visual spatial stimulation and configuration information (Thoma et al., 2014). This gives the right hemisphere an advantage in processing non-verbal, synthetic, and intuitive information. Damage to the right hemisphere tends to cause syndromes with lateral negligence and loss of vision on one side (Mesulam, 1999; Danckert and Ferber, 2006). Additionally, the posterior dorsal parietal lobe participates in storage of spatial working memory, while the frontal lobe played is involved in information processing (Pierard et al., 2011). Finally, activation intensity of the dorsolateral prefrontal cortex increases with increasing working memory load (Chee et al., 2006; Lythe et al., 2012). Taken together, we speculate that spatial tasks in working memory mainly activate the right hemisphere.

Many event-related-potential (ERP) studies have used N2 and P3 waves as a potential physiological indicator to reflect TSD effects. Reduced amplitude and sustained latency of the P3 and N2 component are associated with prolonged sobriety (Koslowsky and Babkoff, 1992; Morris et al., 1993; Jones and Harrison, 2001). A decrease in P3 volatility may reflect a reduction in individual attention and discernment of target stimuli (Zhang et al., 2019). Research in the field of sleep using these techniques has linked TSD with response inhibition and working memory, and shown that working memory would be impaired after TSD. In addition, most of these studies have a pre-post-design (Kjellberg et al., 1977; Venkatraman et al., 2011). However, we know less about whether TSD has an effect on the dominant functional areas of certain cognitive processes, specifically whether a lack of sleep would alter functional asymmetry in spatial working memory. There is a functional compensation mechanism in the brain (Drummond et al., 2004). During language learning tasks, a dynamic compensatory change between the prefrontal cortex and parietal area may occur after sleep deprivation (Drummond et al., 2000). Whether impaired spatial working memory after sleep deprivation is characterized by asymmetry or because of the brain's functional compensation to maintain the original characteristics also needs further discussion.

In this study, we designed a two-back spatial working memory task and used a pre-post-design to explore whether TSD impairs the right-hemisphere advantage of spatial working memory. To examine variation in functional asymmetry after TSD, we recorded electroencephalogram (EEG) data to evaluate changes in N2 and P3 waves. It was hypothesized that there is a right hemisphere advantage in spatial working memory. After sleep deprivation, the spatial working memory capacity is overall impaired, and the right hemisphere advantage is also affected.

# MATERIALS AND METHODS

## Participants

Sixteen healthy right-handed male adults (age range: 21–28 years, mean age: 23 years) participated in the study. All possessed good sleep habits (PSQI $< 5$) and no mental or physical illness. Additionally, they had normal vision or corrected vision $>1.0$, as well as intelligence scores $>$ 110 on the Raven test. An experimenter explained all procedures before the experiment. For 2 weeks, subjects slept regularly for 7–9 h per day. From 48 h prior to the start of the experiment, participants did not smoke, drink alcohol or coffee and did not consume any medication. Written informed consent was obtained from all participants. The experiment was approved by the Ethics Committee of Fourth Military Medical University.

## Experimental Task

All participants were instructed to complete a two-back task for spatial working memory (**Figure 1**). Stimuli are small black squares, shown on a white background ($3 \times 3$ grid) with an approximate visual angle of $0.4 \times 0.4°$ (width: 0.5 cm, height: 0.5 cm). The task involved 122 trials, each presenting the target for 400 ms; the stimulus onset asynchronous (SOA) time with white "+" was 1600 ms. Participants were asked to left-click the computer mouse if the current target matched the stimulus

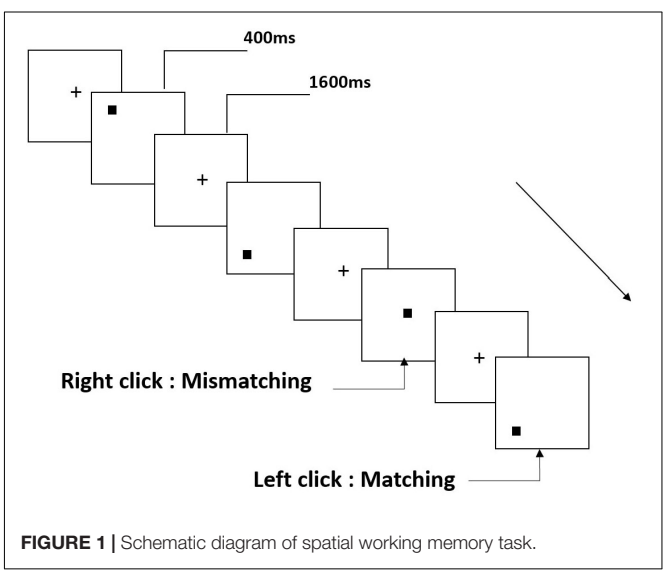


**FIGURE 1 |** Schematic diagram of spatial working memory task.

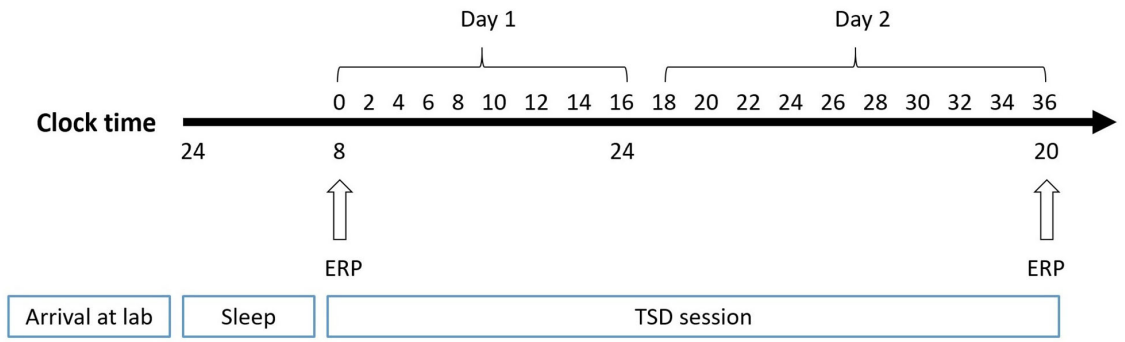


**FIGURE 2 |** Experimental design. After 8 h of sleep in lab, participants experienced 36-h TSD. The arrows indicate the time points during the 2-back spatial working memory task and EEG recording simultaneously.

presented two trials earlier, and right-click if the stimuli did not match. "Matching" and "mismatching" conditions were presented pseudorandomly in a 1:1 ratio.

## Experimental Procedures

The experiment adopted a pre-post design, which is widely used in sleep research. The participants made two visits to the laboratory. In the first visit, they were instructed to practice spatial working memory tasks until they achieved 90% accuracy. Then, they visited the laboratory for a second time the day before the experiment and slept there. At 8:00 AM the next day, subjects performed the spatial working memory task, while experimenters simultaneously recorded the baseline EEG. The second EEG recording was performed after 36 h of TSD in the laboratory (**Figure 2**). During sleep deprivation, central inhibitory or stimulant drugs were prohibited. Subjects were paired and undertook experiments simultaneously. The nursing staff accompanied participants throughout the TSD session for observation and to prevent them from falling asleep.

## EEG Recordings

Continuous scalp EEG was recorded using electrode caps placed at 32 locations of the 10–20 system, along with SynAmps2 amplifiers. The reference is mean mastoids (A1 and A2), and grounded electrodes were placed on the forehead. Recordings occurred at 1000 Hz and all channel impedance was kept below 5 K-Ω. Four additional electrodes were placed above and below the left eye to record bipolar vertical and horizontal electrooculograms.

## Data Analysis of Behavioral Experiments

Data from two subjects were excluded due to technical errors, leaving $n = 14$ for statistical analyses using repeated-measures ANOVA, with Greenhouse-Geisser non-sphericity correction and Bonferroni *post hoc* test. Data are represented as means and standard deviation (SD).

## EEG Data Analysis

Pre-analysis of EEG data was conducted in Scan 4.3. Eye-moment artifacts were corrected with ocular artifact reduction. Epochs with a length of 900 ms (range: −100 ms to 800 ms) at stimulus onset were extracted from EEG data. For each trial, the system automatically rejected voltage exceeding ±100 μV. Epoch data were filtered with a bandpass filter from 0.05 to 30 Hz, and frequency slope was 24 dZB/oct. The components related to the correct response were calculated for further analysis.

Components related to the correct response were calculated. The P3 component was measured as maximum positive values from time windows of 250–450 ms. The N2 component was measured as minimum negative values from time windows of 150–350 ms. Only channels F7, F8, F3, F4, FT7, FT8, FC3, FC4, T7, T8, C3, and C4 were statistically compared here. All ERP results were analyzed using repeated-measures ANOVA to determine the main effects and interactions between deprivation states (baseline and 36-h TSD), hemispheres (left: F7, F3, FT7, FC3, T7, C3 and right: F8, F4, FT8, FC4, T8, C4), and channels (F7, F8; F3, F4; FT7, FT8; FC3, FC4; T7, T8; C3, C4). This also included Greenhouse-Geisser corrections for non-sphericity and Bonferroni *post hoc* tests and simple effects.

# RESULTS

## Behavioral Performance

The results of the behavioral experiments are presented in **Table 1**. The mean reaction time was longer at 36-h TSD than at baseline (ANOVA, $F_{[1,13]} = 1.038$, $P = 0.327$), but the difference was not significant. After 36-h TSD, the accuracy rate of spatial working memory was significantly decreased ($F_{[1,13]} = 10.465$, $P = 0.007$). During 36-h TSD, the main effect of time significantly influenced the correct number per unit time ($F_{[1,13]} = 5.111$, $P = 0.042$).

**TABLE 1 |** Behavioral data (mean ± SD) on the 2-back task at baseline and after 36-h TSD.

| | Baseline | 36-h TSD |
|---|---|---|
| Mean reaction time(ms) | 497.85 ± 82.70 | 520.50 ± 90.91 |
| Correct rate(%) | 0.95 ± 0.04 | 0.86 ± 0.11 |
| Correct number/sec | 1.96 ± 0.33 | 1.72 ± 0.40 |

**TABLE 2 |** The average peak amplitude of the N2 and P3 components in the correct response condition across multiple electrode sites at baseline and after 36-h TSD.

| | | Baseline | | 36-h TSD | |
|---|---|---|---|---|---|
| | | N2 | P3 | N2 | P3 |
| F7 | M ± SD | −3.27 ± 2.55 | 5.44 ± 2.47 | −2.42 ± 2.69 | 2.82 ± 3.44 |
| F8 | M ± SD | −3.23 ± 3.75 | 6.11 ± 3.69 | −2.84 ± 3.38 | 3.63 ± 3.52 |
| F3 | M ± SD | −3.92 ± 4.45 | 7.85 ± 3.77 | −2.63 ± 4.10 | 4.75 ± 4.85 |
| F4 | M ± SD | −3.06 ± 5.05 | 9.46 ± 4.59 | −2.74 ± 4.70 | 5.76 ± 5.24 |
| FT7 | M ± SD | −3.08 ± 2.07 | 6.30 ± 2.68 | −2.02 ± 2.47 | 3.28 ± 4.06 |
| FT8 | M ± SD | −2.91 ± 3.06 | 6.16 ± 3.44 | −2.65 ± 3.26 | 3.33 ± 3.53 |
| FC3 | M ± SD | −3.79 ± 5.99 | 8.58 ± 4.06 | −2.17 ± 4.21 | 6.21 ± 5.05 |
| FC4 | M ± SD | −1.94 ± 5.96 | 10.74 ± 5.15 | −1.68 ± 5.46 | 7.02 ± 5.88 |
| T7 | M ± SD | −2.81 ± 2.22 | 6.94 ± 2.37 | −1.90 ± 2.42 | 4.14 ± 3.55 |
| T8 | M ± SD | −1.97 ± 2.65 | 7.35 ± 3.18 | −1.54 ± 2.56 | 4.51 ± 3.33 |
| C3 | M ± SD | −2.64 ± 6.47 | 9.87 ± 4.14 | −1.84 ± 4.33 | 7.09 ± 4.42 |
| C4 | M ± SD | −1.02 ± 5.25 | 11.36 ± 4.47 | −0.88 ± 4.33 | 8.20 ± 4.85 |

## Amplitude

The means and standard deviations of the amplitudes at the F7, F8, F3, F4, FT7, FT8, FC3, FC4, T7, T8, C3, and C4 electrodes during TSD are listed in **Table 2**.

Compared with baseline, P3 amplitude decreased significantly after 36-h TSD ($F_{[1,13]}$ = 26.880, $P$ = 0.000), but while N2 amplitude decreased after 36-h TSD, the difference was not significant ($F_{[1,13]}$ = 1.064, $P$ = 0.321) (**Table 2**; **Figure 3**). Significant main effects of hemispheres on the P3 amplitude ($F_{[1,13]}$ = 6.077, $P$ = 0.028) were found during the TSD condition (**Figure 4**).

For the P3 amplitude, the interaction between deprivation states (baseline vs. 36-h TSD) and hemispheres (left vs. right) was not significant ($F_{[1,13]}$ = 0.694, $P$ = 0.420). However, simple effect analysis revealed that before sleep deprivation, P3 amplitude was significantly greater in the right hemisphere than in the left ($P$ = 0.016). After sleep deprivation, this asymmetry disappeared ($P$ = 0.128). In addition, we found that the amplitude of P3 was observed most significantly in C3 and C4 ($F_{[5,65]}$ = 33.347, $P$ = 0.000) (**Figure 3**). No other main effects or interaction effects reached statistical significance.

## Latency

The means and standard deviations of the latencies at the F7, F8, F3, F4, FT7, FT8, FC3, FC4, T7, T8, C3, and C4 electrodes during TSD are listed in **Table 3**.

The latency of N2 ($F_{[1,13]}$ = 9.106, $P$ = 0.010) was significantly prolonged after TSD (**Table 3**; **Figure 3**). Although P3 latency was prolonged after 36 h-TSD, the difference was not significant ($F_{[1,13]}$ = 2.734, $P$ = 0.122). In addition, we found the shortest latency of N2 in C3 and C4 ($F_{[5,65]}$ = 4.144, $P$ = 0.002) (**Figure 3**). No other main effects or interaction effects reached statistical significance.

The N2 and P3 amplitudes and latencies that were elicited at the 12 electrode sites are presented in **Figure 3**. The topographic map of the correct response in the working memory task in different sleep conditions (baseline, 36-h TSD and the difference between the two conditions) is presented in **Figure 4**.

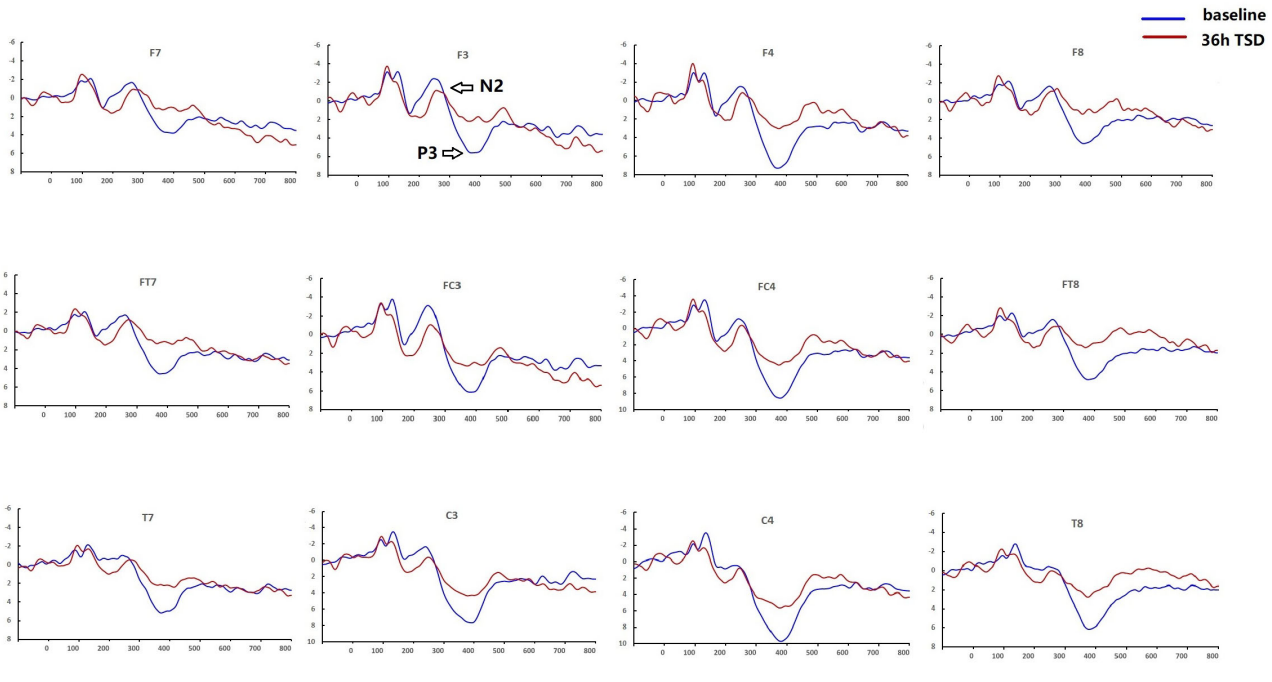


**FIGURE 3 |** Comparison of ERP amplitude and latency between baseline and 36-h TSD for the correct response in working memory. The channels are ordered left-right and top-bottom as follows: F7, F3, F4, F8, FT7, FC3, FC4, FT8, T7, C3, C4, T8.

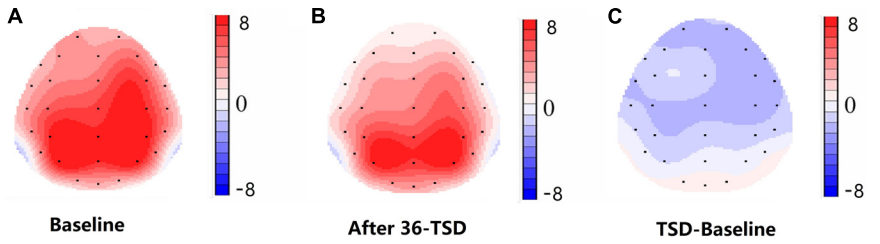


**FIGURE 4 |** Topographic map of correct response during working memory task across different sleep conditions **(A–C)**. **(A)** P3, 250–450 ms, at baseline. **(B)** P3, 250–450 ms, after 36-h TSD. **(C)** P3, 250–450 ms, 36-h TSD baseline subtracted.

**TABLE 3 |** The average peak latency of the N2 and P3 components in the correct response condition across multiple electrode sites at baseline and after 36-h TSD.

| | | Baseline | | 36-h TSD | |
|---|---|---|---|---|---|
| | | N2 | P3 | N2 | P3 |
| F7 | M ± SD | 258.78 ± 32.42 | 386.19 ± 19.63 | 262.25 ± 33.26 | 357.57 ± 36.11 |
| F8 | M ± SD | 248.88 ± 30.21 | 381.27 ± 23.64 | 256.38 ± 23.43 | 376.36 ± 46.88 |
| F3 | M ± SD | 248.81 ± 25.17 | 377.42 ± 25.61 | 270.75 ± 18.97 | 364.46 ± 36.61 |
| F4 | M ± SD | 240.10 ± 26.12 | 372.92 ± 24.58 | 264.88 ± 24.77 | 376.11 ± 34.38 |
| FT7 | M ± SD | 244.88 ± 27.67 | 380.31 ± 26.50 | 270.21 ± 28.03 | 369.86 ± 40.02 |
| FT8 | M ± SD | 249.37 ± 37.18 | 381.46 ± 21.96 | 249.00 ± 33.81 | 360.82 ± 42.98 |
| FC3 | M ± SD | 252.51 ± 17.91 | 372.19 ± 26.02 | 261.17 ± 20.83 | 374.50 ± 29.33 |
| FC4 | M ± SD | 243.42 ± 20.35 | 373.46 ± 28.62 | 266.29 ± 19.65 | 367.54 ± 36.23 |
| T7 | M ± SD | 244.64 ± 21.59 | 379.23 ± 17.48 | 262.79 ± 33.48 | 375.18 ± 29.81 |
| T8 | M ± SD | 242.46 ± 36.71 | 387.50 ± 17.35 | 251.88 ± 35.74 | 362.82 ± 41.29 |
| C3 | M ± SD | 244.85 ± 27.23 | 375.23 ± 27.17 | 246.21 ± 26.78 | 369.36 ± 28.83 |
| C4 | M ± SD | 232.26 ± 23.02 | 371.08 ± 28.84 | 231.75 ± 36.54 | 358.50 ± 39.21 |

## DISCUSSION

In this study, we successfully used ERP analysis to determine the effects of TSD on spatial working memory. Changes in performance during memory tasks were consistent with impaired spatial working memory: participants experienced a decline in accuracy. Prolonged sleep deprivation lowers awakening levels and elevates the awakening threshold, thus hampering work performance (Sanders, 1983; Corsicabrera et al., 1999). Even if an individual remains alert, response time is significantly longer when fatigued than when not fatigued. This phenomenon is related to reduced efficiency in reaction selection and execution after sleep deprivation (Schacht et al., 2008). In this study, the mean reaction time tended to be longer at 36-h TSD than at baseline, but the difference was not significant. When sleep is insufficient, people tend to conservatively estimate their performance. This tendency may increase the likelihood of participating in compensatory behaviors, which may protect against the negative consequences of TSD (Boardman et al., 2018). Furthermore, in terms of ERP indicators, we also observed lower amplitude and greater latency in N2 and P3 waves related to spatial working memory.

The N2 component reflects selective attention and processing of emotional stimuli (Zhang et al., 2014). Here, we demonstrated a significant prolongation of N2 latency, but no significant change in amplitude. Previous results suggest that prolonged N2 latency reflects increased response time after sleep deprivation (Jin et al., 2015). The brain's compensatory functions under limited cognitive resources (Gosselin et al., 2005) may explain the absence of a significant change in amplitude.

We also found that P3 amplitude decreased significantly after 36 h of TSD, consistent with previous results. This decrease confirmed that decision-making in cognitive matching is damaged after sleep deprivation (Panjwani et al., 2010). Sleep deprivation can severely impair continuous attention (Huntley et al., 2011) and other cognitive functions that depend on mental or cognitive abilities (Thomas, 2003; Jarraya et al., 2013). The P3 component reflects deployment of attention resources, and its latency indicates the time window for stimulus classification and evaluation. For example, increased P3 latency is associated with impaired discernment of target stimuli (Kutas et al., 1977). Therefore, we believe that 36-h TSD impairs the individual's spatial working memory ability by affecting the allocation of attention resources, and the individual has a reaction disorder to the change in information (Chua et al., 2014; Honn et al., 2020; Skurvydas et al., 2020). Under baseline conditions, P3 amplitude was significantly greater in the right hemisphere than in the left. This result corroborates the idea that the right hemisphere has a functional advantage when processing spatial information (Li et al., 2016).

Both acute mild sleep loss and extended TSD will undoubtedly cause an increase in sleepiness and decrease in general central

nervous system arousal (Schneider and Fisk, 1984; Cote et al., 2009). Studies have shown that after lack of sleep, individuals' performance in completing the psychomotor vigilance task (PVT) significantly decreases, and both subjective and objective vigilance are negatively affected (Hoedlmoser et al., 2011; Stojanoski et al., 2019). As our results show, after 36 h of sleep deprivation, both behavioral and ERP indicators demonstrated impairment of individual cognitive function. Being awake for a long time changes the activation of the brain's default network and negatively impacts the balance of functional relationship among different brain networks (Wirsich et al., 2018); poor performance after TSD is related to the separation of task goals and inattention (Drummond et al., 2005). Evidence from fMRI suggests that reduced automatic control ability after TSD may be related to a decrease in the activity and/or functional connection of the cerebellar network (Wang et al., 2014). For working memory tasks, the effects of repetitive partial sleep deprivation (PSD) and TSD are not related to the level of execution load, and the effects of PSD are observed to be small (Lo et al., 2012). Although in this study we only selected spatial working memory tasks, sleep deprivation also reduces the quality of information storage in pronunciation working memory (Zhang et al., 2019) and damages the performance of the entire working memory system (Peng et al., 2020).

The disappearance of a right-hemisphere advantage is the principal finding of our study. However, for the amplitude of P3, the interaction between deprivation states (baseline vs. 36-h TSD) and hemispheres (left vs. right) was not significant. In spite of that, from the results of simple effect analysis, we found a right-hemisphere advantage at baseline. However, after sleep deprivation, this asymmetry disappeared. According to the scalp topography, sleep-deprivation-associated changes to the P3 component were greater in the right hemisphere than in the left hemisphere. This outcome may be attributed to the effect that sleep deprivation has on alertness and spatial attention orientation (Mesulam, 1999; Basner and Dinges, 2005). Alertness is a kind of higher internal arousal state obtained and maintained by the individual. The processing of alertness is divided into phasic alertness and continuous tonic alertness (Raz and Buhle, 2006). Extensive research evidence suggests that alertness is mainly controlled by the right frontal parietal cortex (Fan et al., 2002; Petersen and Posner, 2012).Brain injury studies have shown that impaired right hemispheres damage alertness systems more than impaired left hemispheres (Fernandez-Duque and Posner, 2001). Additionally, the two forms of alertness (phasic and tonic) have significant advantages in the right hemisphere of the brain (Sturm et al., 2006; Fan et al., 2009). Neuroimaging and transcranial direct-current-stimulation studies of normal individuals have also revealed that the right hemisphere is more involved in attention distribution to the left and right visual fields (Corbetta et al., 1993; Chambers et al., 2004). Moreover, the ventrolateral frontal cortex is involved in relocating potential stimuli and has a right hemisphere advantage, in contrast with the frontoparietal network responsible for selective attention (Shulman et al., 2010). Visual spatial attention selectively activates the right parietal cortex of the brain (Corbetta, 2000). Considering these findings in combination with our results, we believe that sleep deprivation impairs spatial attention and alertness system, which may be the internal mechanism of spatial working memory damage after sleep deprivation, and the sleep-deprivation-induced impairment of spatial working memory is asymmetric, occurring mainly in the right hemisphere. Although sleep loss of less than 36 h can affect cognition function (Kusztor et al., 2019; Stojanoski et al., 2019), whether it will significantly impair the superiority of the right hemisphere in processing spatial working memory task still needs to be further explored.

Our study has some limitations. First, we only used a two-back task and did not compare working-memory performance across different difficulties (e.g., three- or four-back). Therefore, we cannot address how workload might influence the negative effects of sleep deprivation. Second, because we only used male volunteers, we hesitate to extend our conclusions to women; future research could increase female subjects to study related issues. There are reasons to believe gender differences may exist; fMRI revealed, for instance, that women's left hemisphere is more active when performing spatial memory tasks, whereas men's right hemisphere is more active on the right brain when performing spatial memory tasks, and men use integrated strategies more to help remember spatial locations (Frings et al., 2006). Thus, combining our procedure with fMRI would facilitate a clearer picture of sleep deprivation's effects on working memory. Third, our low sample size may have affected our ability to generate stable results, as we did not find significant differences for N2 amplitude or P3 latency. In addition, due to the limitation of our sample size, we were unable to find any significant interaction effect between deprivation states and hemispheres directly. Finally, circadian biorhythms affect behavioral performance, and these effects differ across individuals (Montplaisir, 1981; Lavie, 2001). We could not rule out effects of circadian rhythms in this study because we did not measure EEG data at the same time points.

## CONCLUSION

In conclusion, our research showed that sleep deprivation impaired spatial working memory, specifically damaging lateralization (i.e., right-hemisphere advantage). Therefore, we recommend that future studies consider brain asymmetry when investigating cognitive deficits associated with sleep deprivation. Further clarity on this issue would improve the development of measures to reduce adverse health effects associated with sleep deprivation. Overall, this study provides valuable physiological evidence for understanding mechanisms underlying the effects of sleep deprivation on spatial working memory.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Fourth Military Medical University. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

YS designed the study. ZP produced the results and wrote the manuscript. CD, XC, LZ, JL, SX, HW, and TY contributed to the data collection and analysis. YS and YW were the guarantors of this study. All authors listed have approved it for publication.

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# Decreased Functional Connectivity Between the Right Precuneus and Middle Frontal Gyrus Is Related to Attentional Decline Following Acute Sleep Deprivation

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**Objectives:** Acute sleep deprivation (SD) seriously affects cognitive functions, such as attention, memory, and response inhibition. Previous neuroimaging studies have demonstrated a close relationship between the functional activities of the precuneus (PC) and the function of alert attention. However, the specific effect of the PC on attention decline after acute SD has not been elucidated. In this study, we used resting-state functional magnetic resonance imaging (fMRI) to study the relationship between the changes of the PC functional connectivity and alertness decline after total SD.

**Methods:** Thirty healthy, right-handed adult men participated in the experiment. Alert attention and functional connectivity were assessed by the Psychomotor Vigilance Test and a resting-state fMRI scan before and after total SD. The region of interest to region of interest ("ROI-to-ROI") correlation was employed to analyze the relationship between the PC and other brain regions after acute SD.

**Results:** Participants showed decreased alert attention after total SD. In addition, SD induced decreased functional connectivity between the right PC and the right middle frontal gyrus (MFG). Moreover, there was a significant correlation between the decreased PC functional connectivity and alertness decline after total SD.

**Conclusion:** Our findings suggest that the interruption of the connection between the right PC and the right MFG is related to the observed decline in alert attention after acute SD. These results provide evidence further elucidating the cognitive impairment model of SD.

**Keywords: attention, functional connectivity, middle frontal gyrus, precuneus, sleep deprivation**

## INTRODUCTION

Modern society and occupational demands have led to increasing sleep deprivation (SD). Due to long and irregular working and studying hours, both adults and adolescents have been getting less sleep over the past three decades (Ford et al., 2015; Keyes et al., 2015; Sheehan et al., 2018; Hisler et al., 2019). Moreover, about 35% of the population sleep less than 6 h/day due to tight work schedules and the use of electronic devices before bedtime. Short duration of sleep is significantly associated with increased mortality (Itani et al., 2017), and SD has significant effects on body function. Some believe that SD influences higher brain functions, such as mood and working memory (Krause et al., 2017; Krause Posada-Quintero et al., 2019), and basic brain functions, such as attention and alertness. SD is not simply a representation of SD and its associated functions; it is also a combination of many harmful factors, such as prolonged insomnia and lack of sleep (Krause et al., 2017).

Extensive research has been carried out on the effects of SD on attentional alertness. Molecules associated with sleep stress, such as adenosine and the hypothalamic system, control the mechanisms involved in the transition between sleep and arousal and are candidates for the chemical signaling and network regulation that typically regulates sleep loss in dose-dependent attention disorders (Saper et al., 2010). Neuroimaging analysis of how acute SD alters brain function related to attention tasks has shown that functional magnetic resonance imaging (fMRI) signals in the dorsolateral prefrontal cortex (DLPFC) and the parietal sulcus were reduced during attention tasks after SD (Chee et al., 2010, 2011; Czisch et al., 2012). In fact, not only did SD reduce task-related activity in these frontal and parietal regions, but it also reduced connections to the lateral visual cortex during visual–spatial attention tasks. In addition, SD affects thalamic activity during sustained attention, suggesting that the thalamus may play an interactive role in the SD-influenced network (Tomasi et al., 2009; Chee et al., 2011). Recently, default mode network (DMN) instability was discovered in the attention deficit associated with SD (Buckner and DiNicola, 2019). Some reports describe the inability of the anterior and posterior cortical regions of the DMN to completely deviate from the midline during the execution of sustained attention tasks under SD conditions. In addition, in the sustained attention test, increased DMN activity during task execution predicted slower execution speed and decreased accuracy of participants (Drummond et al., 2005). In contrast, the significance detection network, including the frontal insular cortex, showed reduced activity during attentional tasks after sleep loss (Ma et al., 2015).

Areas of the brain that belong to the DMN are more susceptible to SD (Chen et al., 2018; Tashjian et al., 2018). It is reported that the precuneus (PC) may be an important "distribution node" in the DMN (Li et al., 2019). Furthermore, based on partial correlation analysis, Fransson pointed out that the PC might be the only network node in the DMN that directly interacts with other nodes (Fransson and Marrelec, 2008). A growing body of other evidence suggests that in clinical and laboratory conditions, the PC is closely related to cognitive functions, such as attentional alertness and neuropsychiatric activities (Cherkassky et al., 2006; Groen et al., 2009). Studies have shown that the PC and the neighboring posterior cingulate cortex are responsible for ongoing information gathering from ourselves and the world around us and automatically distributing it (Cabeza and Nyberg, 2000). In the resting state, the PC and the cingulate cortex, as parts of the DMN, are active and are involved in a wide range of attention processes (Hutchinson et al., 2009; Vogt and Derbyshire, 2009). In addition, the structural and functional connections between the PC and thalamus are consistent with the white matter pathways between the PC and thalamus (Crone et al., 2015; Hannawi et al., 2015; Cunningham et al., 2016). Indeed, the role of the PC is not well established due to the lack of specific studies on its function. As it is located between the somatosensory and visual cortex and has no special functional role, the PC has not been the subject of profound research (Cavanna and Trimble, 2006). Until now, the structural and functional changes of the PC under SD have rarely been studied, and the analysis of functional connectivity of the PC by fMRI and attentional alertness has not been analyzed.

In light of this, we attempted to explore the changes in the functional connections between the PC and other brain regions during SD, as well as the correlation between the changes of functional connectivity and in certain aspects of cognitive functioning. In order to screen and preliminarily verify functional changes in connections between the PC and other brain regions, we designed a variety of Visual Analog Scale (VAS) to initially identify the possible brain functions (Huang et al., 2019) and performed further studies using the Psychomotor Vigilance Test (PVT), which is regarded as a "gold standard" tool to assess for the neurobehavioral consequences of SD (Basner and Dinges, 2011; Basner et al., 2015). Through these methods, we further define the functions of the PC.

## MATERIALS AND METHODS

### Participants

We recruited a total of 30 young adults (30 male, right-handed, age range: 20–30 years) and provided financial compensation for their participation in this study. The subjects were all undergraduates or graduate students. Informed consent forms were signed voluntarily by the participants after the process, risks, and benefits of the study were explained to them in detail. After enrollment, specialist physicians who were qualified to practice medicine in China performed standardized medical examinations on them. The main forms of medical examination included subjective inquiry confirmed by self-report and objective examination (i.e., electrocardiogram, scales, hematological monitoring) to eliminate potential major diseases. The inclusion criteria were: (1) No history of cardiovascular disease, respiratory system, nervous system, infectious diseases, mental disorders, and sleep disorders and (2) Having regular daily life and rest habits without sleep disorders (Pittsburgh Sleep Quality Index scale <7); 1 week before the study began, daily activities of participants were conducted according to the normal routine, and consumption of stimulant drinks and food, such as carbonated drinks, tea, and coffee, was banned, and smoking

was recommended to be avoided. All subjects participated in this study voluntarily and provided written informed consent before participation. This study was approved by the Research Ethics Committee of Beihang University (Beijing, China).

## Behavioral Measures

As an auxiliary means of detection, the VAS, a validated and simple psychometric tool, was used to assess the levels of alertness, anxiety, attention, self-confidence, anger, and nervousness of the participants prior to and after SD (Huang et al., 2019). In order to adapt to the 100-point system of the Chinese people, we adjusted the VAS slightly to an evaluation scale that increased every 10 points and is divided into 10 grades from 0 to 100, corresponding to the 0–10 scale of the standard VAS. Through this, we tried to evaluate this simple measurement method and perform a preliminary assessment.

The PVT–a classic monitoring tool for levels of psychomotor vigilance–was used to measure certain aspects of cognitive functioning reflecting the neurobehavioral consequences of SD (Basner et al., 2011). Subjects were instructed to press the button as soon as they saw a visual stimulus presented at random inter-trial intervals appearing on the screen while trying to minimize error operation. A dot (diameter 3 1/4 cm, viewing angle 1.5 × 1.5°) appeared as a visual stimulus in the center of an LCD screen with 1,024 × 768-pixel resolution (refresh rate, 60 Hz). The red dots appeared pseudo-randomly in the center of the screen, lasting up to 1,000 ms before subjects pressed the button, and disappeared immediately when the button was pressed.

## Procedures

The experiments were conducted in a sleep laboratory at the General Hospital of People's Liberation Army and Institute of Beihang University (Beijing, China), which included a sleep monitoring room with noise less than 30 dB and a daily activity room. During the process of the experiment, the subjects took part in the study in batches of four people each, and two operators monitored the physical and mental states of the subjects and supervised the relevant experimental contents. At 8:00 a.m. on the first day of the experiment, the subjects arrived at the laboratory and wore body-movement watches. They performed daily activities from 8:00 to 20:00, including playing games, reading, talking, sitting for rest, and eating. The collection of experimental data was also accomplished during this time. From 22:00 to 8:00 on the second day, the subjects completed at least 8 h of sleep under the supervision of the operators, with monitoring of body movement during sleep. SD began at 8:00 on day 2 and ended at 20:00 on day 3. During the study, subjects performed normal daily activities and completed relevant experimental data collection. MRI scans began at 20:00 on the third day. During this time, monitoring activities, such as electrocardiography and the subjective assessment of scales, were completed. At the end of the experiment, the subjects were supervised by the experimenters while having a restorative-free sleep in the sleep laboratory. After completing the physical health status assessment on the morning of the fourth day, the subjects left the sleep laboratory at 12:00. During the whole experiment, it was ensured that no less than one operator was medically qualified.

Comparisons before and after the experiment design were carried out with the official start of the test. All subjects underwent scanning twice: one during 36 h of SD and another during rested wakefulness (RW). The two scans were performed at least 3 weeks apart to minimize the possibility of residual SD side effects in participants who had undergone SD scans prior to RW scans. Both scans were performed at the same time of day using the same scanning sequence.

All participants were scanned in a 3.0 T Siemens Magnetom Skyra (Siemens Medical Solutions, Erlangen, Germany) with a standard transmit–receive head coil in the General Hospital of People's Liberation Army. Before the beginning of the scan, the subjects were instructed to prepare for the test (by removing magnetic objects, wearing shoes, and wearing earplugs, for example). The subjects were then instructed to lie supine on the MRI bed, and their heads were fixed with sponge and bandage. At the beginning of each scan, high-resolution T1-weighted structural images (176 slices) and three-dimensional gradient echo images were acquired using the following parameters: repetition time = 2 s, echo time = 30 ms, flip angle = 12°, field of view = 256 mm × 256 mm, matrix = 64 × 64, voxel size = 1 × 1 × 1 mm, and no slice gap. Resting fMRI data were acquired with one run of 8 min (240 images per session) using the following parameters: repetition time = 2 s, echo time = 30 ms, flip angle = 90°, field of view = 256 × 256 mm, matrix = 64 × 64, slice thickness = 3 mm, and slice gap = 1 mm. During the scan, the subjects were asked to close their eyes, keep their heads and body as steady and still as possible, and think about nothing. Data, such as heart rate and breathing, were collected at the same time. It is important for the subjects to remain awake during the scanning process, and so the operators communicated with the subjects through a microphone before each scan to remind them to stay awake. After each scan, the subjects were asked whether they had stayed awake during the scanning process.

## Data Processing

The resting-state fMRI images were preprocessed using SPM 12 software (University College London)[1] and the CONN toolbox software version 18a (Neuroimaging Informatics Tools and Resources Clearinghouse)[2], both of which are cross-platform software based on MATLAB (MathWorks, Inc., Natick, MA, United States). We selected the default preprocessing steps of CONN that include structural translation, segmentation, and normalization; functional realignment and unwarping; functional slice-timing correction; functional indirect segmentation and normalization; and outlier detection and smoothing. The first 10 volumes of the functional images were discarded to ensure the equilibration of the MRI data signal. The functional images were then registered to the middle volume of each subject to measure the degree of head movement, and the rotational and translational motion of subjects was

[1] http://www.fil.ion.ucl.ac.uk/spm/

[2] http://www.nitrc.org/projects/conn

limited to 2° or 2 mm in the *x*, *y*, and *z* axes, respectively. Frames showing more than 2° or 2 mm of head movement from one frame to the next were removed. In head movement processing, the mean frame-wise displacement (FD) was also calculated, and the subjects with the mean FD-Jenkinson >0.2 were excluded (Jenkinson et al., 2002). The structural images were normalized directly to the standard Montreal Neurological Institute (MNI)-152 space using EPI templates, with a voxel size of 3 × 3 × 3 mm. The functional images were normalized to the standard space indirectly using the corresponding structural images by which normalized bias correction was generated. A multiple regression was used to remove nuisance signals from the time series. A full nuisance regression including polynomial detrending in amplitude of low-frequency fluctuations was used to remove nuisance signals from the time series (Woletz et al., 2018). The cerebrospinal fluid signal, white matter signal, whole-brain signal, and six motion parameters were then eliminated. Subsequently, the images were spatially smoothed using a Gaussian filter with the full width at half maximum (FWHM) for 6 mm, and a band pass filter of 0.01–0.08 Hz was used to filter the data temporally (Behzadi et al., 2007; Whitfield-Gabrieli and Nieto-Castanon, 2012).

## Functional Connectivity Analysis

We used the CONN software to study the functional connectivity of the PC with other regions by region of interest to region of interest (ROI-to-ROI) analysis. All ROIs were drawn from Automated Anatomical Labeling (AAL) including 90 cortex ROIs and 26 cerebellar ROIs (Tzourio-Mazoyer et al., 2002). In the first-level analysis, the functional connectivity of different sources was assessed separately for each subject, and the mean time series of the seed point regions of the lateral PC was compared with that of the whole brain to generate a ROI-to-ROI diagram. The data processing method mainly included a general linear model convolved with typical hemodynamic response functions. In the second-level analysis, comparisons were made between subjects [SD > RW (1, −1)] based on a general linear model of random effects, and a seed level correction was performed for multiple comparisons (false discovery rate, $p < 0.05$) (Whitfield-Gabrieli and Nieto-Castanon, 2012).

## Behavioral Correlation Analysis

Visual Analog Scale has a good reliability and validity in the preliminary assessment of psychological states (Miller and Ferris, 1993; Huang et al., 2019), so we initially used VAS as first-level subjective measures of mood change before and after SD. We then used PVT to measure changes in psychomotor vigilance before and after SD. As shorter-duration PVT may be more sensitive to sleep loss, we chose the first 3 min of data as the final data for processing (Loh et al., 2004; Basner and Rubinstein, 2011). In order to further assess the functions of the PC, we used Spearman's rank correlation to calculate the correlation coefficient between VAS changes and functional connectivity before and after SD. We thereafter studied the correlation coefficient between changes in PVT and functional connectivity. The false positive control of analysis was considered significant at $p < 0.05$.

# RESULTS

## Initial Quality Assessment of the Data

During the experiments, no accidents or adverse events occurred, and no subjects were excluded for such reasons. In the preliminary processing of the functional magnetic resonance data, two subjects were excluded from all statistical analyses due to head movement exceeding the mean FD-Jenkinson >0.2 and a large number of frames with head movement greater than 2° or 2 mm. In the evaluation of the VAS, two people were excluded because the data were not saved due to errors in the questionnaire acquisition terminal. In the processing of PVT data, no subjects were excluded. A total of $n = 28$ subjects were included in the functional connectivity analysis. A total of $n = 28$ subjects were included in the behavioral analysis, and a total of $n = 26$ subjects were included in behavioral correlation analysis. The second-level correlation analysis between the change in functional connectivity and VAS included 26 subjects. The correlation analysis of the change in functional connectivity and PVT included 28 subjects.

## Behavioral Results

Demographic and Sleep Quality Index data were collected (**Table 1**). By paired sample *t*-test, the VAS showed significant changes before and after SD in levels of anxiety ($t = 7.641$, $p < 0.001$), attention ($t = -2.87$, $p < 0.008$), self-confidence ($t = 6.986$, $p < 0.001$), anger ($t = 7.486$, $p < 0.001$), and nervousness ($t = -2.069$, $p = 0.049$) of the participants (**Table 1**). PVT monitoring data were also statistically analyzed by paired sample *t*-test, identified significant differences in psychomotor vigilance before and after SD, especially in the mean response time (mean RT), and the fastest 10% fastest response time (Fastest 10% RT), the slowest 10% fastest response time (Slowest 10% RT).

**TABLE 1 |** Demographic data, sleep quality index, and psychological traits ($n = 28$).

| | RW | SD | *t* | *p*-value |
|---|---|---|---|---|
| Ages | 24.48 ± 2.57 | – | – | – |
| Height | 175.93 ± 5.01 | – | – | – |
| BMI | 23.64 ± 1.73 | – | – | – |
| PSQI | 3.37 ± 1.19 | – | – | – |
| Attention (VAS) | 82.69 ± 14.85 | 56.54 ± 19.79 | 7.64 | < 0.001 |
| Anxiety (VAS) | 26.15 ± 15.51 | 35.77 ± 19.22 | −2.87 | 0.008 |
| Vigor (VAS) | 83.46 ± 15.99 | 53.08 ± 19.34 | 6.99 | < 0.001 |
| Self-confidence (VAS) | 84.62 ± 14.76 | 59.62 ± 18.86 | 7.49 | < 0.001 |
| Anger (VAS) | 23.08 ± 13.79 | 30.38 ± 17.77 | −2.07 | 0.049 |
| Nervousness (VAS) | 26.15 ± 18.78 | 33.08 ± 17.15 | −2.09 | 0.047 |
| Mean RT (PVT) | 351.42 ± 35.65 | 373.98 ± 42.05 | −4.84 | 0.001 |
| Fastest 10% RT (PVT) | 284.38 ± 27.18 | 312.58 ± 39.21 | −4.40 | < 0.001 |
| Slowest 10% RT (PVT) | 431.53 ± 39.69 | 447.94 ± 41.64 | −2.05 | 0.05 |
| Lapse probability (PVT) | 5.5 ± 10.49 | 7.11 ± 7.20 | −0.97 | 0.34 |

*RW, rested wakefulness; SD, sleep deprivation; BMI, body mass index; PSQI, Pittsburgh Sleep Quality Index; VAS, Visual Analog Scale; RT, reaction time; PVT, Psychomotor Vigilance Test.*

The functional connectivity between the bilateral PC and the whole-brain ROIs in the RW and SD conditions is shown in **Figures 1**, **2**. CONN software was used to calculate the changes in the functional connections between the PC and other seed regions of the brain before and after SD, and it revealed that the functional connections between the right PC and the right middle frontal gyrus (MFG) lobe were obviously weaken (**Figure 2** and **Table 2**).

In this experiment, there is a positive correlation between attention (VAS) and decrease of functional connectivity by Pearson correlation analysis to explore the possible correlations between VAS and the changes in functional connectivity of the right PC and the right MFG (**Figure 3**). Then, the Pearson correlation analysis between PVT and the changes in functional connectivity of the right PC and the right MFG also suggested that the decreased functional connectivity was significantly negatively correlated with an increased maximum 10% RT of PVT (**Figure 4**).

## DISCUSSION

In this study, we assessed the effects of 36 h of SD on functional connections between the PC and other regions of the brain. The results showed that the functional connections between the right PC and the right MFG were significantly weakened after 36 h of acute SD. However, the changes of functional connectivity between the PC and other brain regions showed no significant statistical changes.

Previous studies have shown that the brain functions sensitive to SD are associated with substantial impairments in cognitive performance (especially attention and working memory), emotion, and regulation and memory abilities

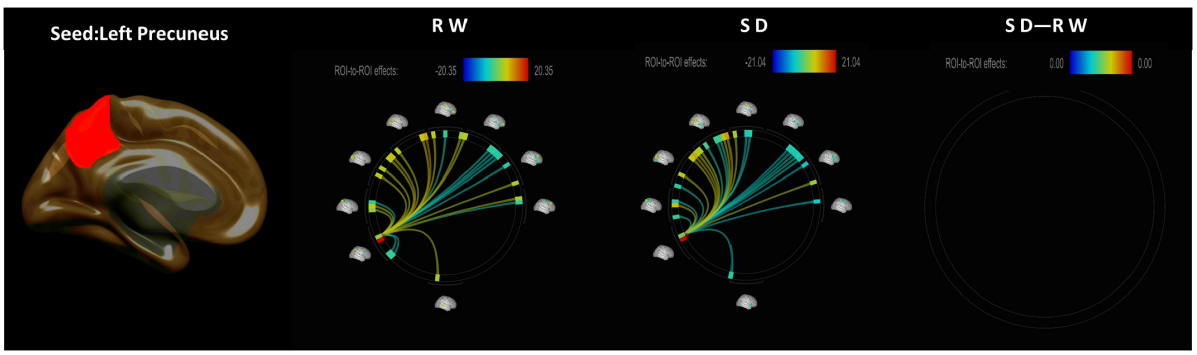


**FIGURE 1 |** ROI-to-ROI functional connectivity of the left precuneus during the RW, SD, and SD > RW conditions. False discovery rate-corrected ($p < 0.05$) for ROI-to-ROI tests. ROI, region of interest; SD, sleep deprivation; RW, rested wakefulness.

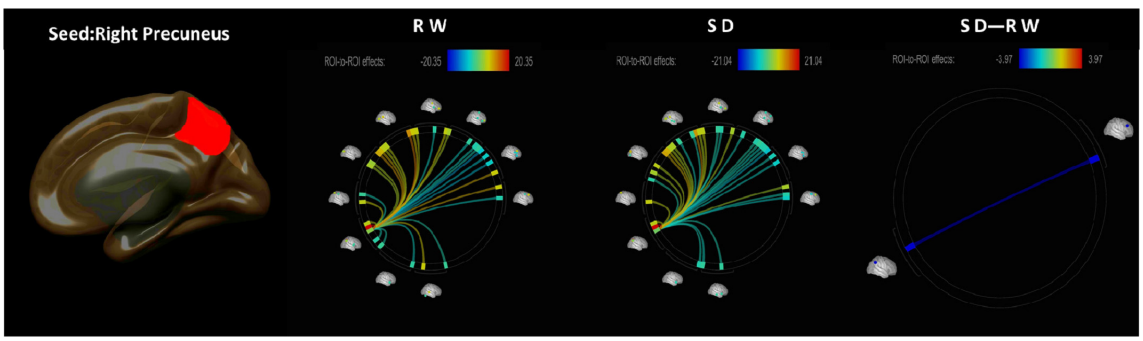


**FIGURE 2 |** ROI-to-ROI functional connectivity of the right precuneus during the RW, SD, and SD > RW conditions. False discovery rate-corrected ($p < 0.05$) for ROI-to-ROI tests. ROI, region of interest; SD, sleep deprivation; RW, rested wakefulness.

**TABLE 2 |** ROI-to-ROI functional connectivity statistics for an individual seed region: comparisons between SD and RW scans (*t*-test).

| Target region | AAL label | MNI center | *t* | Uncorrected *p*-value | FDR-corrected *p*-value |
|---|---|---|---|---|---|
| rPC | Right precuneus | 9, −56, 44 | | | |
| lPC | Left precuneus | −8, −56, 48 | | | |
| Rmfg | Right middle frontal gyrus | 37, 33, 34 | −3.97 | 0.0005 | 0.0324 |

*ROI, region of interest; SD, sleep deprivation; RW, rested wakefulness; AAL, Automated Anatomical Labeling.*

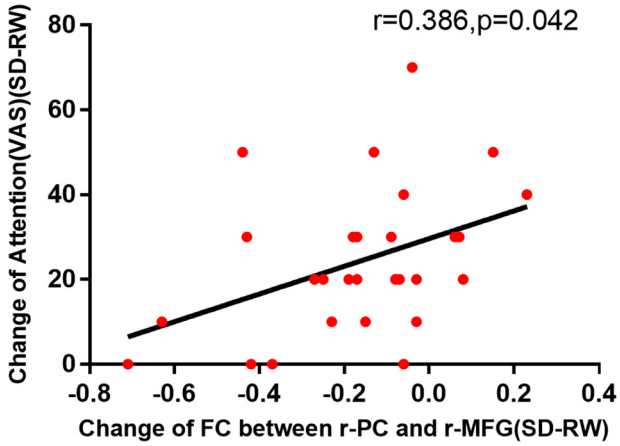


**FIGURE 3 |** The change in functional connectivity was significantly correlated with the VAS of attention. VAS, Visual Analog Scale; SD, sleep deprivation; RW, rested wakefulness.

(Durmer and Dinges, 2005; Goel et al., 2009; Kelley et al., 2013; Louca and Short, 2014). On the one hand, the DMN is closely related to attention task execution under the SD condition. However, the DMN is not a homogeneous network, and SD has a dissociative effect on the functional connectivity of the DMN. The functional connectivity between the ventral DMN and the dorsal DMN is enhanced after SD. After SD, the decrease of functional connectivity of the dorsal DMN is associated with impairment of basic cognitive function and associated with RTs of PVT (Buckner and DiNicola, 2019). In the case of the PC, it is located in the dorsal region of the posterior medial parietal lobe. As a part of the dorsal DMN that is involved in a wide spectrum of attention processes (Vogt and Derbyshire, 2009), the PC acts as an attention information integration center that organizes information from different regions of the brain (Lin et al., 2011). In contrast, some studies suggest that the inferior frontal junction comprising posterior aspects of the inferior frontal sulcus may be an important node that interacts between the ventral attention network (VAN) and the Dorsal Attention Network (DAN) (Asplund et al., 2010). Some studies propose that the right MFG may be the node that links the dorsal and ventral networks (Fox et al., 2006). Interruption of the ongoing process of the dorsal network while focusing on a new task-related external stimulus is not recommended (Corbetta et al., 2008). Furthermore, a positive function of the MFG consisted of attention tasks, suggesting that it is a key feature in sustained attention (Neale et al., 2015), and that the MFG is an important part of the attention network (Gogulski et al., 2017). Given the consistency of multiple functions between the two brain regions and considering that the functional connection between the right PC and the right MFG is the only significant change between the PC and the rest of the brain before and after SD, it is reasonable to presume that the functional connections between the PC and the MFG were probable to be attention-related.

Thereafter, the correlation analysis between the changes of the brain functional VAS and the changes between the right PC and the right MFG preliminary supported this hypothesis and suggested that the changes in attention were significantly correlated with the changes in functional connectivity between the two brain regions (**Figure 2**). VAS is a simple but effective subjective assessment method (Huang et al., 2019). Although the sample size was small and there may be bias, the results it presents provide a relatively clear direction for further research on brain function. Changes in functional connectivity led to no obvious related changes in anxiety, vigor, self-confidence, anger, and nervousness. The PC and MFG are important nodes of their respective brain networks, which are related to a variety of brain functions. The PC is associated with a variety of brain functions, not least because the PC region of the brain is an integrated region of neural networks, especially the DMN, where the brain integrates and distributes signals. The MFG is also associated with a variety of functions, including attention, speech, mood, and wakefulness (Kelley et al., 2013). However, the range of brain functional tasks that may be undertaken by the connection between the right PC and the right MFG will be significantly reduced. This will be attributed to inconsistent bilateral brain function. The left and right MFG have clear functional differences, and the functional asymmetry in MFG is concerned with different brain networks (Song et al., 2019); the left MFG has been found to be associated with working memory, memory retrieval, social perception, and emotional regulation (Zhang et al., 2003; Ochsner and Gross, 2005; Wang et al., 2018). The right MFG plays an important role in sustained attention, and the hemispheric specialization of attention function is caused by the incongruous interhemispheric interaction between the left and right MFG. Therefore, the results from correlation analysis of the changes between various VAS and the functional connectivity preliminarily verified our hypotheses.

To further confirm our inference on the basis of the preliminary results, we used a classic tool for monitoring and evaluating SD and further analyzed the correlation between the PVT and changes in the functional connection between the right PC and the right MFG. We found that there was a moderate correlation between the fastest 10% RT ($r = -0.415$, $p = 0.028$) and the functional connection of two regions and a strong correlation between the mean 10% RT and the functional connection ($r = -0.608$, $p = 0.001$). As a classic detection method of psychomotor vigilance, the PVT measures changes in RT to visual stimuli to measure attention and vigilance (Warm et al., 2008). It is a very sensitive measure of vigilant attention as well as the degree of acute and chronic sleep disorder and circadian misalignment (Goel et al., 2009; Basner et al., 2015). With negligible aptitude and learning effects, the PVT is probably the most widely used measure of alertness (Lim and Dinges, 2008; Basner et al., 2015).

In this study, functional connectivity between the right PC and the right MFG after acute SD was associated with a change in attention VAS scores and a change in RTs in the PVT. This suggested that the right PC and the right MFG were involved in the function of attention in their respective networks, that there

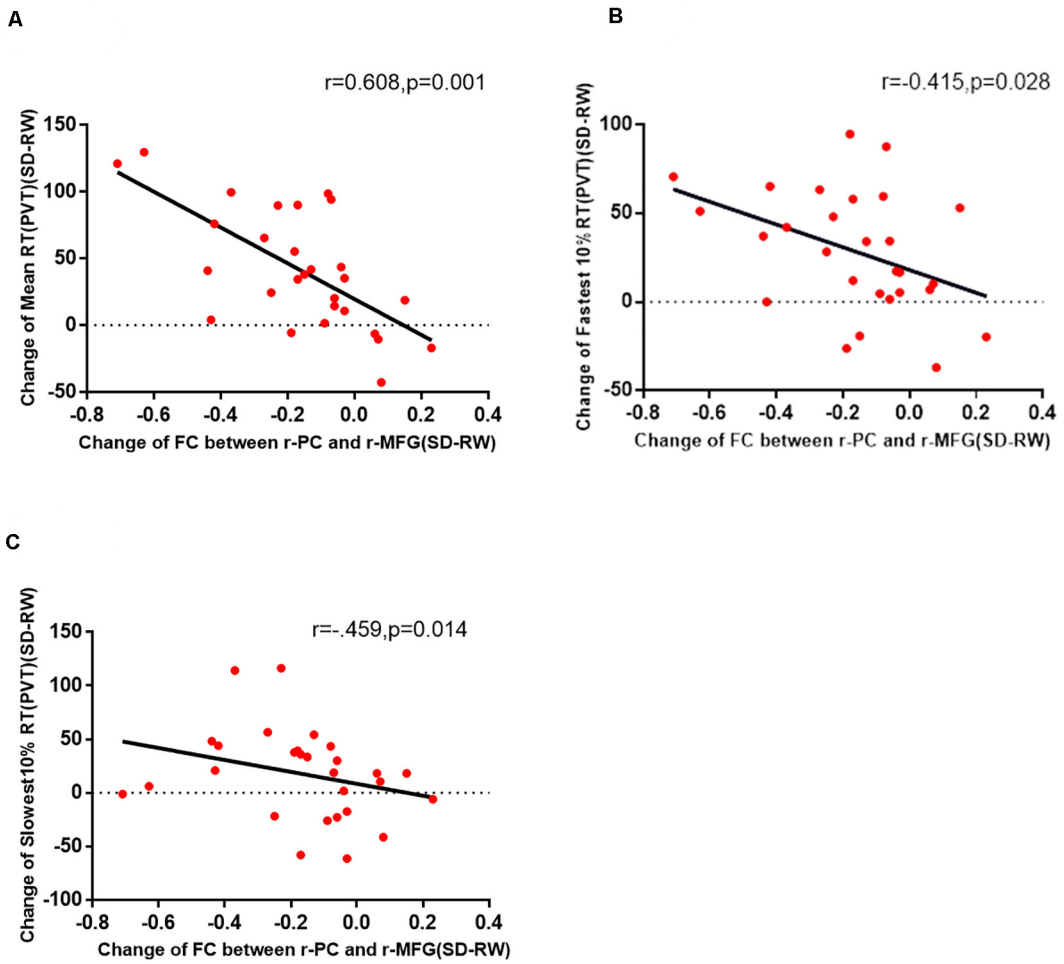


**FIGURE 4 |** The change in functional connectivity between the right PC and the right MFG was significantly correlated with the mean RT **(A)**, fastest 10% RT **(B)**, and slowest 10% RT **(C)** of PVT. PC, precuneus; MFG, right middle frontal gyrus; PVT, psychomotor vigilance test; SD, sleep deprivation; RW, rested wakefulness; RT, response time.

probably exists a connection point between the two networks, and that there was an attention-related brain network connection between the right PC and the right MFG.

## LIMITATIONS OF THE STUDY

Our experiments focused on the effects of acute SD on young people in modern life, given that the majority of work with sleep disorders in China is done by young men. Therefore, the experimental subjects are young men. On the other hand, sleep problems are also prominent in women. We will continue to improve relevant studies in the following studies.

## CONCLUSION

In conclusion, taken together, our resting-state fMRI and behavior results suggest that the functional connections between the right PC and the right MFG before and after SD were decreased, and that the decreased functional connections were significantly correlated with decreased attention. We conclude that the right PC and the right MFG play an important role in the maintenance of attention, and that there may well be a functional connective basis for the maintenance of attention between them, which is an important node for the maintenance of brain attention function.

## DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Beihang University. The patients/participants

provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

BL contributed to performing the experiments and acquisition, analysis, interpretation of the data, and drafted the article. LZ, YZ, and YC contributed to performing the experiments and acquisition of the data. JP reviewed the literature. YS and XZ were the guarantors of this study. All authors have made substantial contribution to this work and approved it for publication.

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# Sleep deprivation induces delayed regeneration of olfactory sensory neurons following injury

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The circadian system, which is essential for the alignment of sleep/wake cycles, modulates adult neurogenesis. The olfactory epithelium (OE) has the ability to generate new neurons throughout life. Loss of olfactory sensory neurons (OSNs) as a result of injury to the OE triggers the generation of new OSNs, which are incorporated into olfactory circuits to restore olfactory sensory perception. This regenerative potential means that it is likely that the OE is substantially affected by sleep deprivation (SD), although how this may occur remains unclear. The aim of this study is to address how SD affects the process of OSN regeneration following OE injury. Mice were subjected to SD for 2 weeks, which induced changes in circadian activity. This condition resulted in decreased activity during the night-time and increased activity during the daytime, and induced no histological changes in the OE. However, when subjected to SD during the regeneration process after OE injury, a significant decrease in the number of mature OSNs in the dorsomedial area of the OE, which is the only area containing neurons expressing NQO1 (quinone dehydrogenase 1), was observed compared to the NQO1-negative OE. Furthermore, a significant decrease in proliferating basal cells was observed in the NQO1-positive OE compared to the NQO1-negative OE, but no increase in apoptotic OSNs was observed. These results indicate that SD accompanied by disturbed circadian activity could induce structurally negative effects on OSN regeneration, preferentially in the dorsomedial area of the OE, and that this area-specific regeneration delay might involve the biological activity of NQO1.

KEYWORDS

**olfactory sensory neuron, olfactory dysfunction, quinone dehydrogenase 1, sleep deprivation, circadian activity**

# Introduction

The mammalian circadian system is essential for alignment of sleep/wake cycles to the 24 h day and for sleep quality (Mohawk et al., 2012). The circadian system controls rhythms in behavior, hormone secretion, and brain metabolism, and modulates the complex multistep process of adult neurogenesis, which is crucial for brain plasticity (Alhola and Polo-Kantola, 2007; Krause et al., 2017). Therefore, its disturbance involves a change in circadian behaviors (Grandin et al., 2006) and could suppress adult neurogenesis, resulting in difficulty in learning and memory (Keisler et al., 2007; Goel et al., 2009; Rasch and Born, 2013).

The olfactory epithelium (OE), inside the nasal cavity, comprises the olfactory sensory neurons (OSNs), which have a special ability to regenerate from progenitor cells throughout life (Mori and Sakano, 2011). Individual OSNs express a single functional allele of one odorant receptor (OR) gene from either the class I or class II repertoires, giving rise to two distinct OR-expressing OSN populations: class I and class II OSNs (Bozza et al., 2009). Class I OSNs are substantially distributed in the dorso-medial region of the OE, and selectively express nicotinamide adenine dinucleotide phosphate H (NADPH) quinone oxido-reductase 1 (NQO1), which is an intracellular enzyme involved in cell protection from natural and exogenous quinones (Gussing and Bohm, 2004). NQO1 is also involved in the regulation of mitotic progression by directly interacting with silent mating-type information regulation (sirt) members, which are a family of signaling proteins involved in metabolic regulation (Chang et al., 2009; Kim et al., 2018; Kang et al., 2018). Because sleep deprivation (SD) significantly downregulates some sirt members and delays neurogenesis in the hippocampus (Chang et al., 2009), regeneration of NQO1-positive OSNs (class I OSNs) that interact with the sirtuin family may likewise be delayed by SD. We thus hypothesized that NQO1-positive and –negative OEs may possess different cell kinetics and exhibit different regeneration processes under SD intervention.

The uninjured OE primarily contains mature OSNs, with a very low rate of OSN regeneration and relatively static cell dynamics (Benson et al., 1984; Farbman et al., 1988). Because of its anatomical location, the OE is directly exposed to environmental agents entering the nasal cavity, leaving OSNs prone to injury. Injury-induced loss of mature OSNs in the OE causes a prompt and massive regeneration of new OSNs through the proliferation and differentiation of progenitor cells (Kikuta et al., 2015).

Mice were subjected to SD over a 2-week period. In addition, the olfactotoxic drug, methimazole, was administered to selectively injure OSNs (Sakamoto et al., 2007). Using these combined methods, the cell dynamics of OSNs following injury were histologically examined under SD intervention.

We found that SD intervention induced a significant decrease in OSNs following injury in the dorsomedial area of the OE, as determined by expression of the NQO1. The NQO1-positive OE exhibited fewer proliferative basal cells and apoptotic OSNs compared to the NQO1-negative OE. These results indicate that SD induce structurally negative effects on the regenerative process, specifically in the dorsomedial area of the OE, and that area-specific injury is involved in the bioactivation of NQO1.

# Materials and methods

## Animals

In this study, 10-week-old male C57BL/6J mice were used (CLEA, Tokyo, Japan). The mice were housed at 22° with a 12-h light and 12-h dark cycle, and given adequate standard pellet food and tap water before all experiments started.

## Sleep deprivation

Mice with modulated circadian activity were created through a two-step process of habituation and SD. The purpose of the habituation period was to familiarize the mice with the wheel environment. During this period, male mice were individually maintained in plastic cages with running-wheels (SW-15S, Melquest, Toyama, Japan) and food access *ad libitum* for 2 weeks under the 12-h light and 12-h dark cycle (**Figure 1A**).

In the SD period, mice were exposed to a continuous stress imposed by the perpetual avoidance of water on a wheel (SW-15-SD, Melquset, Toyama, Japan) (Miyazaki et al., 2013). Briefly, paper-chip bedding was replaced with water to a depth of 1.5 cm. A running-wheel was mounted above the plastic cage, and the mice were forced to stay or run on a running-wheel constantly (**Figure 1A**). Wheel-running activity was continuously recorded at 1 min intervals using the Chronobiology Kit (Stanford Software Systems, Stanford, CA, USA), and activity data are displayed as actograms (**Figure 1D**). To observe the amount of wheel-running activity in a 24-h profile, the wheel activity of each mouse was accumulated in 1 h bins during the 2-week habituation and 2-week SD periods, and then averaged to yield a single 24 h profile (**Figure 1E**).

## Methimazole administration

Methimazole (40 mg/kg; Merck KGaA, Darmstadt, Germany) dissolved in saline was administered intraperitoneally 3 days before the end of the 2-week habituation period to ablate OSNs because the greatest loss of existing OSNs is observed at day 3 post-administration. All mice were sacrificed under deep anesthesia with a combined intraperitoneal injection of ketamine (100 mg/kg) and xylazine (9 mg/kg).

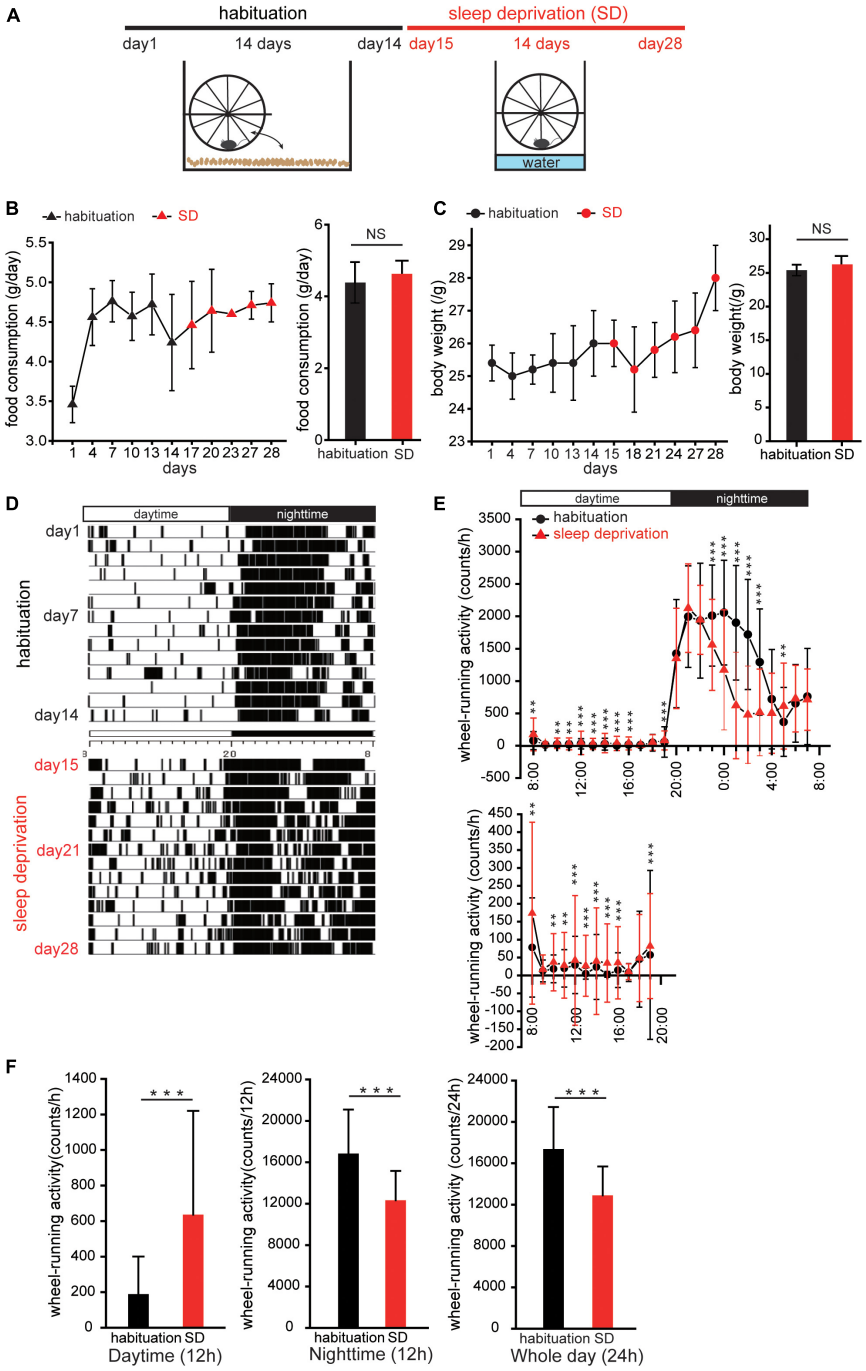


FIGURE 1
Sleep deprivation disturbs circadian activity. **(A)** Protocol for creating mice with disturbed circadian activity. Mice were kept in cages with running-wheels, which they could move freely onto and off in the first 2 weeks (habituation). In the third week, the bedding in each cage was replaced with water to a depth of 1.5 cm. Thereafter, the mice remained on the running-wheel throughout the day to avoid contact with the water (sleep deprivation, SD). **(B,C)** Food consumption and body weights during habituation and SD. Triangles (black, habituation; red, SD) represent changes in food consumption over 28 days; circles (black, habituation; red, SD) represent changes in body weights. No significant differences in food consumption and body weights between habituation and SD periods were observed ($n$ = 5 mice; food consumption, $p$ = 0.13; body weight, $p$ = 0.06; Mann–Whitney $U$ test). **(D)** Representative actogram of wheel-running events during habituation and SD periods. **(E)** Wheel-running activity during habituation (red, triangle) and SD (black, circle) periods plotted over 24 h. Wheel activity in 1 h bins was averaged for 14 days during habituation and SD periods, and the values from individual mice were averaged to yield a single 24 h profile ($n$ = 5 mice). The lower figure shows wheel-running events during the daytime at different scales. *$p$ < 0.05, **$p$ < 0.01, and ***$p$ < 0.001. **(F)** Comparison of wheel-running activity between habituation and SD periods during the daytime (left), night-time (middle), and whole day (right), respectively ($n$ = 5 mice, ***$p$ < 0.001; Mann–Whitney $U$ test).

## Immunohistochemistry

Mice were perfused intracardially with 4% paraformaldehyde in 0.1 M phosphate buffer, decapitated, and postfixed for 24 h in the same fixative. The nasal tissues, including the OE, were decalcified with 10% ethylenediaminetetraacetic acid (EDTA) (pH 7.0) and embedded in paraffin. Coronal sections (4 μm thick) were cut and mounted onto silane-coated slides. The sections underwent immunofluorescence staining as previously described (Tuerdi et al., 2020). Briefly, following deparaffinization and rehydration, sections were immersed in Antigen Retrieval Solution (S1700; Dako Cytomation, Kyoto, Japan) and autoclaved at 121°C for 20 min to allow antigen retrieval to occur. Subsequently, sections were incubated for 20 min with a blocking solution containing 10% bovine albumin serum (Thermo Fisher Scientific, Fremont, CA, USA) to block the binding of non-specific antibodies.

Immunohistochemistry was performed with one or two of the following primary antibodies: anti-olfactory marker protein (OMP, goat polyclonal, 1:3000 dilution; Wako Chemicals, Richmond, VA, USA), anti-NQO1 (rabbit monoclonal, 1:500 dilution; Abcam, Cambridge, MA, USA), anti-sirt2 (rabbit monoclonal, 1:500 dilution; Abcam, Cambridge, MA, USA), anti-activated/cleaved caspase 3 (rabbit polyclonal, 1:500 dilution; Cell Signaling Technology, Danvers, MA, USA), and anti-ki67 (rabbit polyclonal, 1:500 dilution; Thermo Fisher Scientific). Secondary antibodies were donkey anti-goat Alexa Fluor 488 (1:1000; Invitrogen, Eugene, OR, USA) and donkey anti-rabbit Alexa Fluor 594 (1:1000; Invitrogen).

For NQO1 and sirt2 co-staining, a different protocol was required as both antibodies were raised in rabbit. Following deparaffinization, rehydration, antigen retrieval, and antigen blocking, as above, samples were incubated with anti-sirt2 (1:500; Abcam) antibody at 4° overnight. Subsequently, samples were incubated with both donkey anti-rabbit Alexa Fluor 488 at room temperature for 1 h. After washing with PBS several times, the sections were incubated with anti-NQO1 (1:500; Abcam) antibody at 4° overnight before incubation with donkey anti-rabbit Alexa Fluor 594.

## Analysis

For each OE, three coronal sections located between the caudal and the rostral OE regions were examined, and each section was cut at 500 μm intervals. The number of OSNs labeled by anti-OMP, anti-NQO1, anti-activated caspase-3, and anti-Ki67 antibodies was quantitatively analyzed using sections with single or double immunostaining for each antigen and counterstaining with DAPI. Any immunostaining that exceeded two standard deviations (SDs) of mean background intensity of the connective tissue under the lamina propria was considered positive. In each section, three OE areas (each, 100 μm) from both the left and right NQO1-positive OEs and two areas each from the left and right NQO1-negative OEs were arbitrarily selected, and the number of immunostained cells (OMP-, NQO1-, caspase- 3-, and ki67-positive cells) was measured. Data were quantified as the number of positive cells per 100 μm OE. All values are shown as mean ± SD, and the analysis for immunostained cells was performed using ImageJ software (NIH).

## Enzyme-linked immunosorbent assay

Blood cortisol level was determined using a Mouse DetectX Cortisol ELISA Kit (Arbor Assays, MI, USA), according to the manufacturer's instructions. Blood samples were collected under deep anesthesia with a combined intraperitoneal injection of ketamine (100 mg/kg) and xylazine (9 mg/kg).

## Western blotting analysis

Olfactory mucosa was collected from normal, injury only, and injury + SD mice under deep anesthesia with a combined intraperitoneal injection of ketamine (100 mg/kg) and xylazine (9 mg/kg). OE protein purification was performed with a Nucleo Spin RNA/Protein purification kit according to the manufacturer's instructions (NucleoSpin, Machrrey-Nagel GmbH & Co., Düren, Germany). Total proteins were quantified by using bicinchoninic acid (BCA) Protein Assay Kit (Pierce, Thermo Fisher Scientific, Fremont, CA, USA). And 30 μg of protein sample was loaded on a 10% SDS-PAGE gel (e-PAGEL HR, ATTO, Motoasakusa, Tokyo, Japan) along with a protein marker, separated at 135 V, 35 mA in MOPS SDS running buffer (Invitrogen, Fremont, CA, USA) for 80 min, and then electro-transferred (320 mA for 7 min) onto a polyvinylidene fluoride membrane (*Trans*-Blot Turbo Transfer System Transfer Pack, Bio-Rad Laboratories, CA, USA). Samples were blocked in 2% skim milk in Tris-buffered saline/Tween (TBS/T) buffer for 30 min at room temperature. The anti-sirt2 antibody (rabbit monoclonal, 1:1000 dilution; Abcam) was used to detect the sirt2 protein in the OE, and an anti-β-actin antibody (rabbit polyclonal, 1:2000 dilution; Medical & Biological laboratories Co., Tokyo, Japan) was used as an internal reference. Membranes were incubated with the anti-sirt2 antibody or anti-β-actin antibody overnight at 4°, washed several times in TBS/T buffer, and incubated with anti-rabbit horseradish peroxidase (HRP)-conjugated antibody (rabbit monoclonal, 1:3000 dilution; Cytiva, Tokyo, Japan) for 1 h at room temperature. All washes were performed in TBS/T for 4 × 5 min. The blots were imaged using the western blotting detection reagents (Amersham ECL Prime Western Blotting Detection Reagents, Cytiva).

## Statistical analyses

The data were statistically evaluated in Origin Pro software (Origin Lab Corporation, Northampton, MA, USA) and JMP Statistical Discovery software (SAS Institute Japan, Tokyo, Japan). Data in Figures 1B,C,E,F, 2E, 3H, were analyzed using the Mann–Whitney *U*, and the Steel–Dwass test was used to analyze data in Figures 2D, 3G,I, 4B, 5B. A $p$-value of $< 0.05$ was considered statistically significant.

## Study approval

All animal studies were approved by the Experimental Animal Research Committee at the University of Tokyo and carried out in accordance with the approved guidelines.

# Results

## Sleep deprivation disturbs circadian locomotor activity

Mice were kept in cages with running-wheels, which they could move freely onto and off, for a 2 week period to acclimatize animals to the running-wheel environment (habituation, Figure 1A; Miyazaki et al., 2013). In the third week, the bedding in each cage was replaced with water to a depth of 1.5 cm. Thereafter, the mice remained on the running-wheel constantly to avoid contact with the water [SD, Figure 1A]. This method easily allowed for a sustained stress load on the mice over a 2-week period. Food consumption and body weight changes were measured to determine whether stress-induced overeating or weight gain was present (Figures 1B,C). No significant changes in food consumption and body weight were observed in the SD period compared to the habituation period (body weight: $n = 5$ mice, $p = 0.06$; food consumption: $n = 5$ mice, $p = 0.13$, Mann–Whitney *U* test; Figures 1B,C). These results suggest that SD intervention does not induce body weight gain and potentially has no influence on energy metabolic systems.

Comparison of actograms of the habituation period and the SD period (Figures 1D,E, respectively) demonstrated that during the daytime of the SD period, wheel-running activity was typically higher than in the habituation period, while during the night-time, wheel-running activity was typically lower in the SD period than in the habituation period ($n = 5$ mice, $^{**}p < 0.05$, $^{***}p < 0.001$, Mann–Whitney *U* test, Figure 1E). When analyzed separately for the daytime and night-time, wheel-running activity during the daytime of the SD period was significantly greater than in the habituation period, while during the night-time, wheel-running activity was significantly lower in the SD period

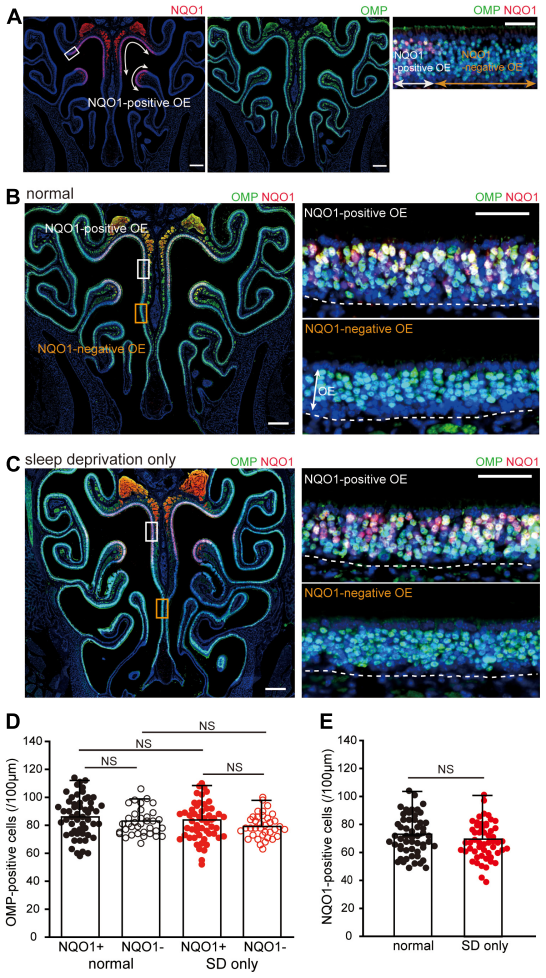


FIGURE 2
Sleep deprivation (SD) does not induce histological changes in the OE. **(A)** Photomicrographs of representative coronal sections from normal mice stained with anti-NADPH quinone oxido-reductase 1 (NQO1) antibody (left, red) and anti-olfactory marker protein (OMP) antibody (middle, green). Mature olfactory sensory neurons (OSNs) were evenly distributed within the OE, but NQO1-positive OSNs were confined to the upper nasal septum and upper concha bullosa, as indicated by the arrow range. Left and middle images, lower magnification; right image, higher magnification image of the area indicated by the square in the left image. Scale, 300 μm at low magnification; 50 μm at high magnification. **(B,C)** Photomicrographs of representative coronal sections from normal **(B)** and SD mice **(C)** stained with anti-OMP antibody (green) and anti-NQO1 antibody (red). Images on the right show higher magnification images captured from the areas indicated by the squares on the image on the left (upper, NQO1-positive OE; lower, NQO1-negative OE). The dashed line represents the basement membrane of the OE. Scale, 300 μm at low magnification; 50 μm at high magnification. OE, olfactory epithelium. **(D)** Counts of OMP-positive cells of NQO1-positive (NQO1+) and NQO1-negative (NQO1 –) OEs in normal and SD only mice (SD only). SD did not induce changes in the number of OMP-positive cells between the NQO1-positive and –negative OEs. NS, not significant; Steel–Dwass test. **(E)** Counts of NQO1-positive cells in normal (black) and SD only mice (red). Significant changes were not observed histologically between normal and SD only mice (NS, not significant, Mann–Whitney test).

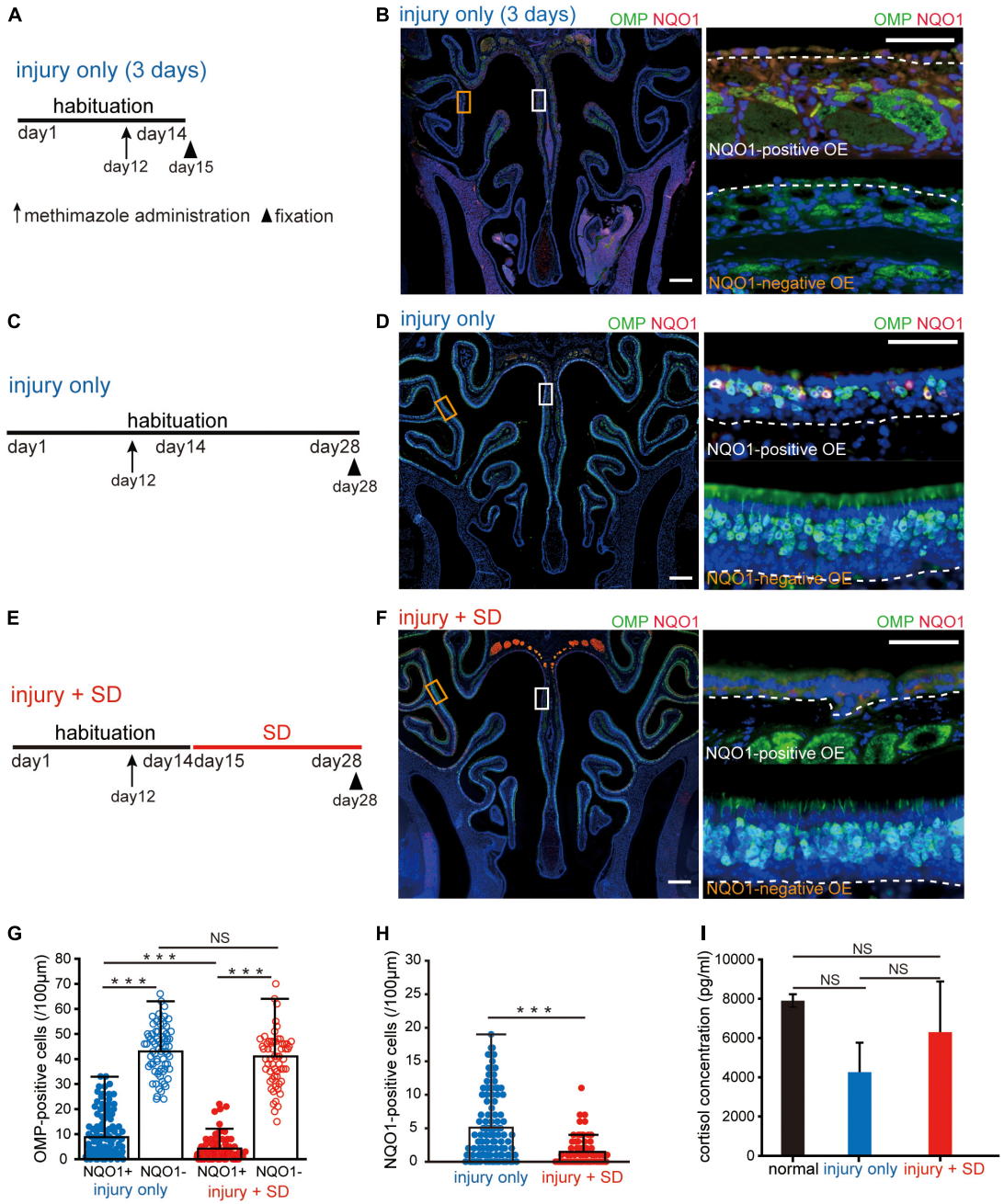


FIGURE 3
Short-term sleep deprivation selectively induces delayed regeneration in the NADPH quinone oxido-reductase 1 (NQO1)-positive olfactory epithelium (OE) following injury. **(A)** Time course and experimental design. Methimazole was administered on day 12, and perfusion with a fixative was conducted on day 15. Arrow indicates the timing of methimazole administration, and arrowhead indicates the timing of fixation. **(B)** Photomicrographs of representative coronal sections captured 3 days after methimazole administration (injury only, 3 days). Pictures on the right show higher magnification images captured from the areas indicated by the squares on the left picture (upper, NQO1-positive OE; lower, NQO1-negative OE). The dashed line represents the basement membrane of the OE. Scale: 300 μm at low magnification, 50 μm at high magnification. **(C)** Time course and experimental design. Methimazole was administered on day 12, and perfusion with a fixative was performed on day 28. **(D)** Photomicrographs of representative coronal sections at 16 days following methimazole administration (injury only). Pictures on the right show higher magnification images captured from the areas indicated by the squares on the left picture (upper, NQO1-positive OE; lower, NQO1-negative OE). Scale: 300 μm at low magnification, 50 μm at high magnification. **(E)** Time course and experimental design. Methimazole was administered at day 12 (arrow) after starting habituation. Three days after methimazole administration, a 2-week SD was initiated, and fixation was conducted on day 28 (arrowhead). **(F)** Photomicrographs of representative coronal sections following methimazole-induced injury and sleep deprivation (injury + SD). Pictures on the right show higher magnification images captured from the areas indicated by the squares on the left picture (upper, NQO1-positive OE; lower, NQO1-negative OE). Scale: 300 μm at low magnification, 50 μm at high magnification. **(G)** Counts of olfactory marker protein (OMP)-positive cells of NQO1-positive (NQO1+) and NQO1-negative

(Continued)

FIGURE 3 (Continued)
(NQO1 –) OEs in injury only and injury + SD mice. In injury only mice, the number of OMP-positive olfactory sensory neurons (OSNs) in the NQO1-positive OE is significantly lower than in the NQO1-negative OE. In mice with injury combined with SD, the number of OMP-positive cells in the NQO1-positive OE was lower than that in the NQO1-negative OE. However, the degree of reduction was significantly greater in the NQO1-positive OE when mice were exposed to a 2-week SD compared with injury only mice (***$p < 0.001$; NS, not significant; Steel–Dwass test). **(H)** Counts of NQO1-positive cells in injury only and injury + SD mice. The number of the NQO1-positive OSNs in mice with injury combined with SD was significantly lower than the number of the NQO1-positive OSNs in injury only mice (***$p < 0.001$; Mann–Whitney *U* test). **(I)** Comparison of blood cortisol levels among normal, injury only, and injury + SD mice. No significant changes in cortisol levels were observed among normal, injury only, and injury + SD mice (NS, not significant, Steel–Dwass test).

than in the habituation period ($n$ = 5 mice, ***$p < 0.001$, Mann–Whitney *U* test, Figure 1F). For the entire day, daily (24 h) wheel-running activity during the SD period was significantly less than in the habituation period ($n$ = 5 mice, ***$p < 0.001$, Mann–Whitney *U* test, Figure 1F). These results suggest that SD intervention disturbs circadian locomotor activities.

## Sleep deprivation for 2 weeks does not induce histological changes in the olfactory epithelium

We next examined the effect of SD on tissue homeostasis in the uninjured OE. When coronal sections of the OE were immunostained with anti-NQO1 antibody (Figure 2A, left) and anti-OMP antibody, to allow identification of mature OSNs (Figure 2A, middle), mature OSNs were evenly distributed across the OE, while NQO1-positive OSNs were limited to the upper nasal septum and upper concha bullosa (indicated by the arrow range in Figure 2A, left). Moreover, NQO1-positive and –negative OEs could be easily distinguished (Figure 2A, right).

When colocalization of OMP and NQO1 was compared in normal mice, no difference in the number of OMP-positive OSNs was seen between the NQO1-positive and –negative OE ($n$ = 3 mice per group; normal: NQO1-positive OE vs. NQO1-negative OE, $p$ = 0.11; Mann–Whitney *U* test; Figures 2B,D). Under SD intervention, there was no significant difference in the number of OMP-positive OSNs between the two different OEs (SD only: NQO1-positive OE vs. NQO1-negative OE, $p$ = 0.65; Steel–Dwass test; Figures 2C,D). Furthermore, the number of OMP-positive OSNs in SD only mice was not significantly different from that in normal mice in both the NQO1-positive and –negative OEs [NQO1-positive (normal) vs. NQO1-positive (SD only), $p$ = 0.48; NQO1-negative (normal) vs. NQO1-negative (SD only), $p$ = 0.76; Steel–Dwass test; Figure 2D]. No significant differences were observed in NQO1-positive OSNs between normal and SD only mice ($n$ = 3 mice per group; normal vs. SD only, $p$ = 0.14; Mann–Whitney *U* test; Figure 2E). These results indicate that although the expression pattern of NQO1 is different, the number of mature OSNs in each OE is equally distributed, and their numbers are not affected by a 2-week SD intervention.

## Sleep deprivation for 2 weeks selectively induces delayed regeneration following injury in NADPH quinone oxido-reductase 1-positive olfactory epithelium

Next, we examined whether the NQO1-positive OE showed histological changes in response to SD intervention during the regeneration process following injury. During the first 2 weeks after OE injury, differentiation of basal progenitor cells is markedly enhanced to allow the number of OSNs to recover to pre-injury levels (Kikuta et al., 2015). We therefore focused on the regeneration of OSNs during the first 2 weeks following injury, when cellular dynamics are likely to be greatest.

Firstly, the OE was histologically evaluated 3 days after administration of the olfactory toxic drug methimazole [injury only (3 days), Figure 3A]. Analysis of coronal sections of the injured OE immunostained with anti-OMP and anti-NQO1 antibodies (Figure 3B) demonstrated that, with the exception of basal progenitor cells, almost all OSNs undergo cell death, and that both the NQO1-positive and –negative OE were uniformly injured (Figure 3B). These results suggest that the stage for differentiation of OSNs is reset 3 days after injury, providing an opportunity to study the kinetics of cells within the OE. We next examined the effects of SD on regeneration of OSNs following injury (Figure 3C). Figure 3D shows a representative coronal section of the OE immunostained with anti-OMP and anti-NQO1 antibodies 16 days following injury. In the NQO1-negative OE, the number of OSNs returned to pre-injury levels, but in the NQO1-positive OE, the OE was thinner and had fewer OSNs.

Next, mice were exposed to a 2-week SD at 3 days following injury (methimazole-induced injury plus SD; injury + SD, Figure 3E). In injury only mice, the number of OMP-positive OSNs in the NQO1-positive OE was significantly lower than in the NQO1-negative OE ($n$ = 5 mice per group; injury only: NQO1-positive OE vs. NQO1-negative OE, ***$p < 0.001$; Steel–Dwass test; Figures 3F,G). These results suggest that the degree

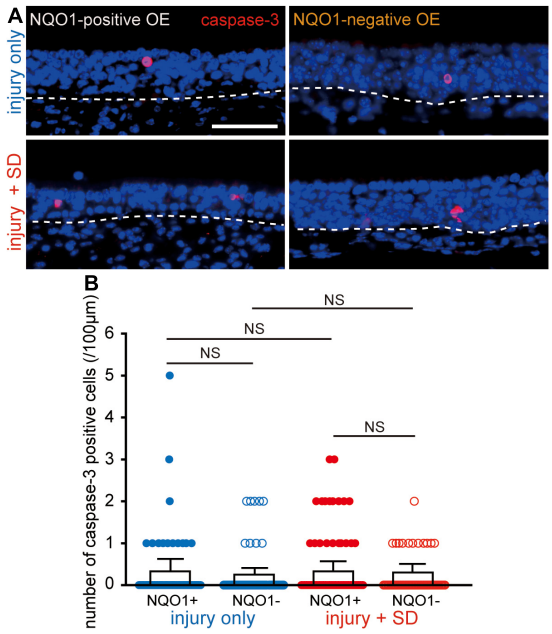


FIGURE 4
Neither injury only nor injury combined with sleep deprivation (SD) induces apoptosis. **(A)** Coronal sections stained with anti-caspase-3 antibody in the NQO1-positive (left), and –negative OE (right) in mice with injury only (upper, injury only) and mice with injury combined with SD (lower, injury + SD). The dashed line represents the basement membrane of the olfactory epithelium (OE). Scale, 50 μm. **(B)** Counts of cleaved caspase-3-positive cells in the NQO1-positive and –negative OE, in injury only and injury + SD mice. There was no significant change in the number of caspase-3-positive cells seen in the NQO1-positive OE in mice with injury combined with SD compared with that in mice with injury only. NS, not significant; Steel–Dwass test.

of regeneration following the injury is not uniform within the OE.

In mice with injury combined with SD, the number of OMP-positive OSNs in the NQO1-positive OE was significantly lower than that in the NQO1-negative OE (injury + SD: NQO1-positive OE vs. NQO1-negative OE, $^{***}p < 0.001$; Steel–Dwass test; Figure 3G). However, the degree of reduction was significantly greater in the NQO1-positive OE when mice were exposed to a 2-week SD [NQO1-positive (injury only) vs. NQO1-positive (injury + SD), $^{***}p < 0.001$; Steel–Dwass test; Figure 3G]. No significant difference in the number of OMP-positive cells was observed in the NQO1-negative OE between injury only mice and injury + SD mice [NQO1-negative (injury only) vs. NQO1-negative (injury + SD), $p = 0.16$; Steel–Dwass test; Figure 3G]. Furthermore, the number of the NQO1-positive OSNs was significantly lower in mice with injury combined with SD than in injury only mice ($n = 5$ mice per group; injury only vs. injury + SD; $^{***}p < 0.001$; Mann–Whitney $U$ test; Figure 3H). These results suggest that SD intervention accompanied by the disturbed circadian locomotor activities enhances the delay in regeneration following injury, but the effect is limited to a specific area within the OE.

Various negative regulators of regeneration could be affected by stress, and SD is often perceived as a physiological stressor (Inoué et al., 1995). Thus, the effect of SD on regeneration following injury in the NQO1-positive OE may be caused by excessive stress. We therefore assessed the degree of stress by measuring blood cortisol levels. No significant changes in cortisol levels were observed among normal, injury only, and injury + SD mice ($n = 5$ mice per group; normal vs. injury only, $p = 0.19$; injury only vs. injury + SD, $p = 0.55$; normal vs. injury + SD, $p = 0.98$; Steel–Dwass test; Figure 3I). These results indicate that the reduction in the number of OMP-positive OSNs in the NQO1-positive OE was indeed due to an effect of SD intervention, rather than excessive stress associated with experimental manipulation.

## Sleep deprivation does not increase apoptotic olfactory sensory neurons in the NADPH quinone oxido-reductase 1-positive olfactory epithelium

The reduction in the number of OSNs in the NQO1-positive OE by intervening SD may be due either to an increase in OSN cell death, a decrease in the number of newly generated OSNs, or both. To examine these possibilities, we determined the expression of active caspase-3 in the OE under the two different experimental conditions (injury only, and injury + SD; Figure 4) using an antibody specific for the active (cleaved) form of caspase-3, a marker of apoptotic cell death that functions in most downstream caspase-activation cascades (Yuan et al., 2003).

Figure 4A shows representative images stained with anti-caspase-3 antibody of mice with injury only (upper, injury only) and mice with injury combined with SD (lower, injury + SD), in the NQO1-positive and –negative OEs (left, NQO1-positive OE; right, NQO1-negative OE). In injury only mice, there was no change in the number of caspase-3-positive cells between NQO1-positive and NQO1-negative OEs ($n = 5$ mice per group; injury only: NQO1-positive OE vs. NQO1-negative OE, $p = 0.99$; Steel–Dwass test; Figure 4B). In mice with injury combined with SD, a similar trend between the NQO1-positive and NQO1-negative OE was also observed (injury + SD: NQO1-positive OE vs. NQO1-negative OE, $p = 0.93$; Steel–Dwass test; Figure 4B). Furthermore, the number of caspase-3-positive cells in the NQO1-positive OE in mice with injury combined with SD was not significantly higher than in mice with injury only [NQO1-positive (injury only) vs. NQO1-positive (injury + SD), $p = 0.46$; Steel–Dwass test; Figure 4B], and similar trends were observed in the NQO1-negative OE [NQO1-negative (injury only) vs. NQO1-negative (injury + SD), $p = 0.96$; Steel–Dwass

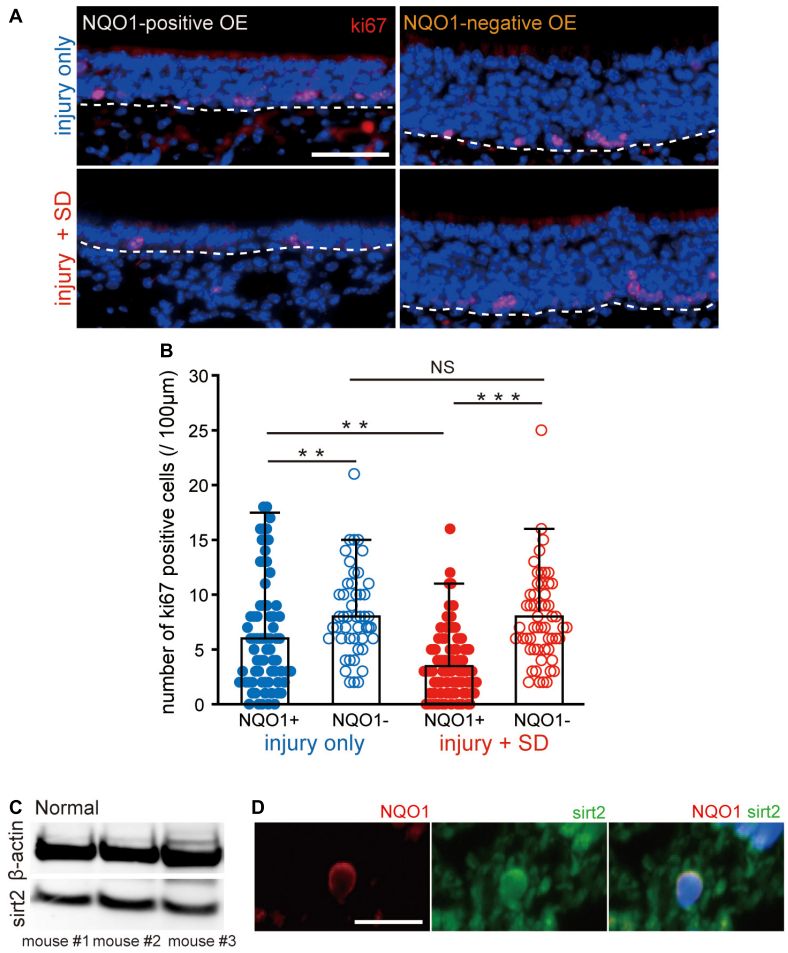


FIGURE 5
Sleep deprivation induces downregulation of proliferation in the NADPH quinone oxido-reductase 1 (NQO1)-positive olfactory epithelium (OE) following injury. **(A)** Coronal sections stained with anti-ki67 antibody in the NQO1-positive (left) and −negative OEs (right), in mice with injury only (upper, injury only) and mice with injury combined with sleep deprivation (SD) (lower, injury + SD). The dashed line represents the basement membrane of the OE. Scale, 50 μm. **(B)** Counts of ki67-positive cells in the NQO1-positive and −negative OE, in injury only and injury + SD mice. Injury only mice exhibited fewer ki67-positive cells in the NQO1-positive OE than in the NQO1-negative OE. In mice with injury combined with SD, the number of ki67-positive cells was significantly lower in the NQO1-positive OE compared with the NQO1-negative OE. The extent of the reduction in the NQO1-positive OE in injury + SD mice was greater than that in injury only mice. ***$p < 0.001$, **$p < 0.05$, NS, not significant, Steel–Dwass test. **(C)** Detection of sirt2 protein in the OE. Total protein from 30 μg weight olfactory mucosa was separated and analyzed for sirt2 and β-actin by western blotting. The anti-β-actin antibody was used as an internal reference. **(D)** Coronal sections stained with anti-NQO1 (red) antibody and anti-sirt2 (green) antibody in mice with injury combined with SD. NQO1-positive cell was co-stained with an anti-sirt2 antibody. Scale, 20 μm.

test; Figure 4B]. These results argue against the hypothesis of upregulation of cell death in the NQO1-positive OE.

## Sleep deprivation induces downregulation of proliferation in olfactory sensory neurons in NADPH quinone oxido-reductase 1-positive olfactory epithelium

The delayed regeneration observed in the NQO1-positive OE may result from decreased OSN proliferation. To examine the extent of OSN proliferation, we examined expression of the cell proliferation marker, ki67, in the NQO1-positive and −negative OEs (Figure 5). Figure 5A shows representative images stained with anti-ki67 antibody of sections obtained from mice with injury only (upper, injury only) and mice with injury combined with SD (lower, injury + SD) in the NQO1-positive and −negative OEs (left, NQO1-positive OE; right, NQO1-negative OE). In injury only mice, the proportion of ki67-positive cells in the NQO1-positive OE was significantly lower than in the NQO1-negative OE ($n = 5$ mice per group; injury only: NQO1-positive OE vs. NQO1-negative OE, **$p < 0.05$; Steel–Dwass test; Figure 5B). Similarly, in mice with injury

combined with SD, there were fewer ki67-positive cells in the NQO1-positive OE than in the NQO1-negative OE (injury + SD: NQO1-positive OE vs. NQO1-negative OE, $^{***}p < 0.001$; Steel–Dwass test; **Figure 5B**). Furthermore, the extent of the reduction in injury + SD mice was greater than in injury only mice [NQO1-positive (injury only) vs. NQO1-positive (injury + SD), $^{**}p < 0.05$; Steel–Dwass test; **Figure 5B**]. By contrast, no significant difference in the number of ki67-positive cells of the NQO1-negative OE was observed between injury only mice and injury + SD mice [NQO1-negative (injury only) vs. NQO1-negative (injury + SD), $p = 0.90$; Steel–Dwass test; **Figure 5B**]. Taken together, these results suggest that SD intervention does not increase apoptotic OSNs in the NQO1-positive OE, but instead leads to delayed regeneration due to a marked reduction in mitosis in progenitor basal cells in the NQO1-positive OE.

NADPH quinone oxido-reductase 1 regulates mitotic progression through modulation of sirt2 activity in the hippocampus (Chang et al., 2009; Kim et al., 2018). We therefore examined whether sirt2 is also expressed in the OE. As expected, western blot analysis demonstrated that sirt2 was expressed in the OE of three mice (**Figure 5C**). Furthermore, immunofluorescence staining demonstrated that sirt2 was co-expressed with NQO1 in OSNs (**Figure 5D**). These results suggest that the NQO1 expressed in the OE may regulate mitotic progression through interaction with sirt2.

## Discussion

In this study, we have examined how SD intervention affects the maturation of new OSNs following methimazole-induced injury. This method also necessarily involves some stress load. However, the method used in this study was found not to be an excessive stress load that would be accompanied by an increase in cortisol. SD mice demonstrated decreased locomotor activity during the night-time and increased activity during the daytime. Changes in circadian activity for 2 weeks did not cause histological changes in the OE. However, during the repair process following chemically induced OE injury, changes in circadian activity delay OSN regeneration, selectively in the NQO1-positive OE, which represents proliferating cells. Furthermore, in this region, while there was a decrease in the number of proliferative basal cells, no changes in apoptotic OSNs were observed. These results indicate that circadian activities play an important role in regenerative process preferentially in the NQO1-positive OE, and that their disturbance causes a delay in the incorporation of new OSNs into the neural circuit.

Olfactory sensory input is an important factor involved in regeneration of the OE. After methimazole-induced damage to the mouse OE, when olfactory input is blocked by silicon tube insertion, new OSNs fail to mature and instead undergo apoptosis (Kikuta et al., 2015). Insulin signaling is also a necessary factor for tissue regeneration. In diabetic mice, decreased insulin signaling prevents new OSNs from differentiating into mature OSNs, resulting in reduced olfactory function (Kuboki et al., 2021). In addition to these factors, sleep could play an important role in regeneration for some areas of the OE, and ensuring sleep without circadian rhythm disruption may be one of the factors necessary for functional recovery in the olfactory system.

Sleep disorder is defined by irregular circadian rhythm including difficulties in falling and/or staying asleep, followed by functional impairment while awake. We created SD mice under a weak stress continuously imposed throughout the day and night by the perpetual avoidance of water on a wheel to induce disturbed circadian locomotor activity, based on a previously reported technique (Miyazaki et al., 2013). Mouse models of sleep disorders have historically been based on SD techniques. Typical methods of inducing chronic SD include the classic disk-over-water technique and the use of slowly rotating wheel (Lopez-Rodriguez et al., 2004; Kim et al., 2007). These methods force mice to remain awake, and thus cannot be maintained for periods of 48 h or repeated for 20 h/day for 5 days because mice survivability decreases (Lopez-Rodriguez et al., 2004; Kim et al., 2007). Temporal exposure to conventional stressors, such as immobilization or electric shock at fixed times of the day, affects sleep time and consolidation (Pawlyk et al., 2008), while the timing of stress load provides mice with zeitgeber cues that induce stress anticipation activity (Barnum et al., 2007). Furthermore, in these model mice, 6–8 weeks of stress exposure gradually reduces gross and nocturnal locomotor activity, but does not affect diurnal locomotor activity. Thus, it does not affect circadian locomotor activity throughout the day and night. An environment in which the animal is placed in the territory of a male rat of the same species for an hour, which is social defeat stress, also rapidly suppresses nocturnal locomotor activity, but does not increase daytime activity (Meerlo et al., 1996).

One drawback of the SD method used here is its lack of versatility, as only a limited number of mouse strains have been examined for its effects on autonomic nervous system and hormone secretion (Miyazaki et al., 2013). In addition, this method uses the hedonistic nature of wheel running, a rewarding activity. Thus, it may be difficult to sustain a disturbance in circadian locomotor activity over a long period of time, as the mouse may become less hedonistic without appropriate rest.

In the C57BL/6 mice used in this study, the 2-week wheel environment did not cause changes in diet or body weight, but there are inter-strain differences in response to changes in environmental conditions. For example, in C3H/He mice, during 2 weeks of SD, food intake increases but body weight gradually decreases (Miyazaki et al., 2013). In general, C3H/He mice tend to exhibit more anxiety, depression-like behaviors, and stress responsivity compared with C57 BL/6 mice (Kopp et al., 1999). These differences in behavior and autonomic responses among mouse strains may have different effects on OE

regeneration as well as changes in body weight and food intake after 2 weeks of SD.

The negative effects of continuous stress load have been hypothesized to be related to the cellular consequences of prolonged waking, including cell toxicity by excess glutamate release, free radicals, or elevated glucocorticoids, all of which may affect cell kinetics in the OE (Inoué et al., 1995; Mamelak, 1997; Guzmán-Marín et al., 2003). However, a previous report indicates that SD within 14 days does not increase apoptotic cell death, as determined by TUNEL in many brain regions (Cirelli et al., 1999). Similarly, we failed to observe a significant increase in active caspase-positive cells in the NQO1-positive OE compared to the NQO1-negative OE. Furthermore, we observed no significant increase in blood corticosterone levels under SD intervention, indicating that it is unlikely that the delayed regeneration in the NQO1-positive OE observed in mice with injury combined with SD is primarily due to excessive stress load. This suggests that other factors may be responsible for the adverse effects of SD.

Cell proliferation is regulated by several factors, including endogenous substances and inflammatory cytokines (Gould et al., 1999; Tanapat et al., 1999; Cameron and McKay, 2001; Kempermann, 2002). These factors are substantially affected by SD, and may provide a link between insufficient sleep and reduced neurogenesis. For instance, insulin-like growth factor, (IGF)-1, is one of several growth factors known as neurogenesis promoters (Trejo et al., 2001), but long-lasting SD in rats resulted in lower IGF-1 binding (Everson and Crowley, 2004). The brain-derived neurotrophic factor (BDNF) facilitates hippocampal neurogenesis (Scharfman et al., 2005; Guzman-Marin et al., 2006), and the hippocampal expression of BDNF was decreased after 48 h of SD (Guzman-Marin et al., 2006). Furthermore, exposure to IL-6 and TNF-a, which are increased after chronic SD, diminishes cell proliferation *in vitro* (Irwin et al., 2006; Haack et al., 2007). Thus, these factors may be one reason for delayed regeneration across the OE. However, they cannot explain the heterogeneous regeneration mechanisms observed within the OE such that SD delays regeneration of the NQO1-positive OE.

NADPH quinone oxido-reductase 1 plays multiple physiological roles as a consequence of its ability to directly interact with the sirt family members (Gaikwad et al., 2001; Kang et al., 2018). Indeed, NQO1 regulates mitotic progression by functioning as an NAD(P)H dehydrogenase through modulating sirt2 activity (Chang et al., 2009; Kim et al., 2018). Consistently, we observed coexpression of NQO1 and sirt2 in OSNs. The response to mitotic stress also involves sirt1, through its binding to NQO1 and its activation (Chang et al., 2009). Thus, the NQO1-positive OE could possess a cell proliferation mechanism that strongly depends on the interaction of NQO1 with sirt family members, suggesting that some sirt family members are downregulated under SD accompanied by disturbed circadian activity, selectively delaying regeneration of NQO1-positive OE.

A possible reason for delayed regeneration in the NQO1-positive OE in the absence of SD may be related to the different expression patterns of all-*trans*-retinoic acid (RA) in horizontal basal cells (HBC), one of the neurogenic stem cells. RA is a morphogen derived from Vitamin A, which regulates organogenesis and tissue regeneration, and is widely used to induce differentiation of pluripotent stem cell cultures (Gudas and Wagner, 2011). HBCs in the NQO1-positive OE differ significantly from those in the NQO1-negative OE with regard to RA availability (Login et al., 2015). RA bioavailability in NQO1-positive HBCs is low compared with NQO1-negative HBCs because of increased expression of the RA-degrading enzyme, cytochrome P450 family 26 (Håglin et al., 2020). Thus, NQO1-positive HBCs following injury may exhibit lower proliferative potential than NQO1-negative HBCs, even without SD intervention.

Although olfactory disorders have a wide variety of causes (Jones and Rog, 1998; Murphy et al., 2002; Brämerson et al., 2004), treatments to achieve complete tissue regeneration and functional recovery have not been well established. It is interesting from a therapeutic perspective to speculate that SD triggers reduced expression of the sirt family in the OE. Initial drug development efforts focused on sirt1 and sirt2 have yielded several promising activators that have now been through the first clinical trials, with evidence of safety and efficacy (Dai et al., 2018). Thus, sirt family members may become an attractive therapeutic target, potentially leading to the development of new agents for loss of OSNs in the NQO1-positive OE.

The axons of class I OSNs (NQO1-positive OSNs) are converged onto the dorsomedial and anteromedial region of the olfactory bulb (OB) (a dorsal domain for class I odorant receptors, $D_I$ domain), while the axons of class II OSNs (NQO1-negative OSNs) are converged onto the dorsolateral, anterolateral (a dorsal domain for class II odorant receptors, $D_{II}$ domain), and ventral region of the OB (a ventral domain for class II odorant receptors, V domain) (Mori and Sakano, 2011). This class-specific anatomical domain organization in the OB correlates with functional odor-induced innate responses (Mori and Sakano, 2011). The projection of class I OSNs to the $D_I$ domain is responsible for innate aversive behavior to odorants produced from the spoiled foods, while the projection of class II OSNs to the $D_{II}$ domain is responsible for innate fear responses to predator odors (Kobayakawa et al., 2007; Mori and Sakano, 2011). Furthermore, it has been reported that olfactory behaviors are altered by the biased OR class choice of OSNs (Enomoto et al., 2019). Class II-dominant mice created by genetic manipulation of the transcription factor, Bcl11b, exhibited the same reduction in aversion to odorants produced from spoiled foods and predator odors as the wild type, indicating that behavioral outputs toward two distinct aversive odorants depend on the populations of class I and class II OSNs. In wild mice, an equilibrium between the proportion of class I and class II OSNs allows for high sensitivity and

subsequent appropriate reactions to danger-signaling odors, while an imbalance in the proportion of class I and II OSNs could have serious negative consequences for adaptation to the external environment and survival of the species.

There is increasing evidence that the circadian system regulates the multi-step process of adult neurogenesis through rhythmic systemic factors such as neurotransmitters, hormones, and intrinsic factors such as redox status and clock genes/molecular clocks within neural progenitor cells (Ali and von Gall, 2022). SD accompanied by disturbed circadian activity have been suggested to be a potential risk factor for the development of Alzheimer's disease and related dementias and Parkinson's disease, in addition to being a symptom of neurodegeneration (Leng et al., 2019). However the mechanistic link between circadian rhythms and neurodegeneration is still not fully understood. Therefore, a better understanding of how circadian activity modulates adult neurogenesis in various brain regions including the OE may provide a key to elucidate the pathophysiology of neurodegenerative diseases as well as olfactory disorders.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Ethics statement

The animal study was reviewed and approved by the Experimental Animal Research Committee at the University of Tokyo.

## Author contributions

BH and SK designed the studies, wrote the manuscript, and created all drawings in figures. BH, SK, and TK performed the experiments. BH analyzed the data. BH, SK, TK, KK, and TY revised and finalized the manuscript. All the authors read and approved the manuscript.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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# Causal association of sleep disturbances and low back pain: A bidirectional two-sample Mendelian randomization study

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**Background:** Previous observational studies have shown that low back pain (LBP) often coexists with sleep disturbances, however, the causal relationship remains unclear. In the present study, the causal relationship between sleep disturbances and LBP was investigated and the importance of sleep improvement in the comprehensive management of LBP was emphasized.

**Methods:** Genetic variants were extracted as instrumental variables (IVs) from the genome-wide association study (GWAS) of insomnia, sleep duration, short sleep duration, long sleep duration, and daytime sleepiness. Information regarding genetic variants in LBP was selected from a GWAS dataset and included 13,178 cases and 164,682 controls. MR-Egger, weighted median, inverse-variance weighted (IVW), penalized weighted median, and maximum likelihood (ML) were applied to assess the causal effects. Cochran's *Q* test and MR-Egger intercept were performed to estimate the heterogeneity and horizontal pleiotropy, respectively. Outliers were identified and eliminated based on MR-PRESSO analysis to reduce the effect of horizontal pleiotropy on the results. Removing each genetic variant using the leave-one-out analysis can help evaluate the stability of results. Finally, the reverse causal inference involving five sleep traits was implemented.

**Results:** A causal relationship was observed between insomnia-LBP (OR = 1.954, 95% CI: 1.119–3.411), LBP-daytime sleepiness (OR = 1.011, 95% CI: 1.004–1.017), and LBP-insomnia (OR = 1.015, 95% CI: 1.004–1.026), however, the results of bidirectional MR analysis between other sleep traits and LBP were negative. The results of most heterogeneity tests were stable and specific evidence was not found to support the disturbance of horizontal multiplicity. Only one outlier was identified based on MR-PRESSO analysis.

**Conclusion:** The main results of our research showed a potential bidirectional causal association of genetically predicted insomnia with LBP. Sleep improvement may be important in comprehensive management of LBP.

KEYWORDS

sleep disturbance, low back pain, Mendelian randomization, causal effect, insomnia

## Introduction

Low back pain (LBP) is a common disease and an important factor leading to limited activity, absenteeism, and disability (Woolf and Pfleger, 2003; Hartvigsen et al., 2018). In the National Health Interview Survey (NHIS), 31,044 participants were questioned and more than 25% of people stated they had experienced at least 1 day of LBP in the past 3 months (Deyo et al., 2006). A specific age limit does not reportedly exist for the occurrence of LBP. The incidence of LBP peaks in the third decade of life, and the prevalence increases until 60–65 years of age and then gradually declines (Golob and Wipf, 2014). Simultaneously, severe pain can reduce physical activity and may cause chronic musculoskeletal pain (Cimmino et al., 2011) which can significantly reduce the quality of life and social productivity of patients, resulting in high social costs. According to a rough estimate, the economic burden of LBP in Great Britain was close to 300 million pounds (Schofield et al., 2012). Unfortunately, the pathogenesis of LBP remains unclear and some scholars suggested it may be due to the interaction of biological, psychological, and social factors (Lall and Restrepo, 2017). Gender, obesity, aging, smoking, and mood disorders are several confirmed risk factors for LBP (Parreira et al., 2018; Shiri et al., 2019; Bento et al., 2020). Furthermore, sleep disturbances and the prognosis of patients with LBP has been an area of increased interest in recent years.

The potential association between sleep disturbances and LBP has been reported in many studies (O'Donoghue et al., 2009; van de Water et al., 2011; Murase et al., 2015). LBP was shown independently associated with short sleep duration and poor sleep quality (Murase et al., 2015). In another observational cohort study of 761 patients with LBP (Pakpour et al., 2018), after controlling for confounders such as depression, the researchers found that sleep disturbance was a risk factor for pain intensity which decreased after the sleep disorders were resolved. In addition, sleep condition and pain intensity were assessed in 80 patients with LBP. Generalized estimation equation (GEE) analysis showed that poor sleep quality and lower sleep efficiency may increase pain intensity the next day (Alsaadi et al., 2014). However, the above-mentioned observational studies did not yield a clear conclusion and unpredictable confounders might have produced reverse causality.

Mendelian randomization (MR) referred to a novel method which evaluate the causal association between a modifiable exposure and a clinically relevant outcome (Sekula et al., 2016). There were inherent defects in observational researches, and the presumption of the conclusions needs to strictly ensure the control of potential confounders. Considering that the alleles of genetic variants associated with exposure were randomly assigned, the performance of MR could effectively save time and economic costs, and help researchers explore the causal effect between exposures of interest and the outcome. Therefore, we conducted a bidirectional two-sample MR analysis to explore the causal relationship between the different traits of sleep disturbance and LBP.

In the present study, we hypothesized a causal relationship may exist between sleep disturbances and LBP. Although LBP has been primarily treated with analgesics, the application of sleep regulators may have potential usefulness in the comprehensive management of LBP.

## Materials and methods

### Data sources

In order to comprehensively assess the causal relationship between sleep disturbance and LBP, we selected five traits (insomnia, sleep duration, short sleep duration, long sleep duration, and daytime sleepiness) that can reflect sleep disturbance as genetic variants (Jia et al., 2022; Ni et al., 2022).

Insomnia is a common clinical condition characterized by difficulty initiating or maintaining sleep, accompanied by symptoms such as irritability or fatigue during wakefulness (Buysse, 2013). In the present study, the genetic variants of insomnia were obtained from the genome-wide association

Abbreviations: LBP, low back pain; CLBP, chronic low back pain; GEE, generalized estimation equation; GWAS, genome-wide association studies; SNP, single nucleotide polymorphism; IVs, instrumental variables; LD, linkage disequilibrium; IVW, inverse-variance weighted; MR, Mendelian randomization; ML, maximum likelihood; OR, odds ratio; 95% CIs, 95% confidence intervals; NO, nitric oxide; nNOS, neuronal nitric oxide synthase; ESZ, eszopiclone; PAG, periaqueductal gray matter; MT, membrane receptors; NMDA, *N*-Methyl-D-aspartate.

study (GWAS) among 336,965 individuals of European ancestry from the UK Biobank.

Other sleep traits such as sleep duration, short sleep duration, and long sleep duration, were described in detail in another GWAS summary statistics involving 128,266 subjects (Jones et al., 2016). Sleep duration was recorded based on self-reported sleep time and subjects who reported more than 18 h of sleep within 24 h were excluded from this study. Then, adjustment for age, gender, and study center was made to obtain the model residuals, and inverse normalizing was performed to ensure a normally distributed phenotype. Descriptions of short sleep duration and long sleep duration were defined based on average sleep duration. Short sleep duration refers people who slept less than 6 h per day on average and 28,980 subjects were included in this group. Long sleep duration reported an average of more than 9 h of sleep per day and 10,102 subjects were included in this group. GWAS summary statistics of daytime sleepiness were derived from the UK Biobank (Wang et al., 2019). A total of 452,071 participants of European genetic ancestry self-reported the frequency of daytime sleepiness using the question: "How likely are you to doze off or fall asleep during the daytime when you don't mean to? (e.g., when working, reading or driving)," with the answer categories "never" ($N$ = 347,285), "sometimes" ($N$ = 92,794), "often" ($N$ = 11,963), or "all of the time" ($N$ = 29). All participants who had taken any sleep-related medication were excluded. The latest GWAS summary statistics describing LBP (finn-b-M13_LOWBACKPAIN) was obtained by visiting the website[1]. This GWAS dataset consisting of 13,178 cases and 164,682 controls from European ancestry was identified in 2021.

## Selection of instrumental variables

Researchers screened the genetic variants that met the conditions based on strict quality control from the GWAS summary statistics of various sleep traits including insomnia, sleep duration, short sleep duration, long sleep duration, and daytime sleepiness. It was worth to emphasize that when performing MR analysis using genetic variants (usually single nucleotide polymorphisms [SNPs]) as instrumental variables (IVs), the IVs also need to satisfy three core assumptions: (1) Genetic variants are strongly associated with exposure factors; (2) Genetic variants are associated with the outcome only through the exposure of interest; and (3) Genetic variants are not associated with other confounders affecting the outcome (Boef et al., 2015). First, the single nucleotide polymorphisms (SNPs) associated with five sleep traits with genome-wide significance ($p$ < 5e-8) were extracted. To obtain more IVs associated with the exposure of interest, relaxed thresholds, setting the maximum threshold to 5e-6, were used. This approach to threshold relaxation has been reported in other studies (Chen et al., 2020; Kwok and Schooling, 2021). Because the existence of linkage disequilibrium (LD) may cause corresponding bias, controlling LD before subsequent analysis was necessary. In this study, independent SNPs were selected by setting $r^2 < 0.001$ and window size = 10,000 kb.

To further understand whether selected genetic variants were associated with potential confounders and the outcome, researchers visited PhenoScanner[2], a website which provides details about genetic variants and phenotype information. We focused on physical and psychosocial factors including obesity, smoking, and mood disorders (e.g., anxiety and depression) associated with LBP. IVs that were significantly associated with the above confounders were eliminated before proceeding. However, because genetic variants were not readily available, this process was not performed under extremely stringent conditions.

## Mendelian randomization analysis

Statistical analyses were conducted using the R programming language (version 4.0.5). MR analysis was performed based on the "TwoSampleMR" package (version 0.5.6), and the "MRPRESSO" package (version 1.0) was used to apply MRPRESSO analysis to identified the outliers.

Various MR analysis approaches including MR-Egger, weighted median, inverse-variance weighted (IVW), penalized weighted median and maximum likelihood (ML) were performed to estimate the casual effects between sleep traits (insomnia, sleep duration, short sleep duration, long sleep duration, and daytime sleepiness) and LBP. The unbiased estimate of the causal effect can be obtained by IVW regression due to the non-existence of horizontal pleiotropy (Davies et al., 2019). The results of IVW analysis were considered as the major outcome. IVW was able to combine the effect of individual SNP on the outcome and obtained the ratio estimates (β). The ratio estimates were converted to acquire the corresponding odds ratios (ORs) and 95% confidence intervals (95%CIs). MR-Egger, which performing the directional pleiotropy, test of causal effect and the estimate of the causal effect, was an analytical method for MR using pooled genetic summary data (Burgess and Thompson, 2017). The pleiotropy of genetic variants may lead to the failure of the core assumption of IVs, but the causal effect can still be more accurately calculated when up to 50% of the information comes from invalid IVs in the method of weighted median estimator (Bowden et al., 2016). Both of the MR-Egger regression and weighted median could perform to improve the evaluation of IVW due to the robust estimates they could offer. Other methods such as ML (Hartwig et al., 2017)

1 https://gwas.mrcieu.ac.uk/datasets/

2 http://www.phenoscanner.medschl.cam.ac.uk/

and penalized weighted median were mainly used to assess the robustness of MR results.

## Heterogeneity and horizontal pleiotropy

The horizontal pleiotropy of genetic variables is important because the results of MR analysis can be significantly affected and cause instability in the effect estimates. The test of correlation horizontal pleiotropy was mainly evaluated based on MR-Egger intercept and MR-PRESSO analysis; the former estimated the possibility of horizontal pleiotropy by calculating the term of intercept obtained after linear regression analysis. *F* statistics were calculated to evaluate the strength of the IVs. MR-PRESSO analysis identifies the outliers that may possess the characteristic of horizontal pleiotropy. The number of distributions in MR-PRESSO analysis was set to 1,000, and the robustness of MR analysis results was evaluated by comparing whether the casual relationship was affected before and after the outlier elimination. IVW and MR-Egger regression were applied to test the heterogeneity and Q statistics were calculated to quantitatively evaluate the heterogeneity. If the heterogeneity existed ($p < 0.05$), then the results of random effect IVW were dominant, otherwise it referred to the results of fixed effect IVW.

## Data visualization

To further evaluate whether there was a single SNP with a large contribution of pleiotropy that may cause deviation to the results, leave-one-out analysis was conducted to eliminate SNPs one by one and then re-estimate the causal effect. The forest plot was used to evaluate the effect estimation between the genetic variants and LBP, and MR-Egger regression and IVW to calculate the combined effects. If a correlation between parts of the SNPs and LBP was found, a specific rule determined whether a related SNP should be eliminated. More specifically, if $p_{exposure} > p_{outcome}$, then the SNP was not included in the MR analysis. Funnel plot was used to evaluate the publication bias and applied to assess the potential directional pleiotropy in this study.

# Results

## Selection of instrumental variables

**Supplementary Tables 1–3** shows the process of IV filtering in detail. SNPs were removed in the following situations. First, in the process of extracting SNPs from the outcome GWAS dataset, parts of the SNPs not found in the outcome dataset were removed. The results obtained from analysis of the four groups were as follows: insomnia-LBP (rs11804386), sleep duration-LBP (rs282086 and rs12537376), short sleep duration-LBP (rs573615914, rs546786239, rs8008258, and rs74500417), and long sleep duration-LBP (rs2387776, rs73196898, rs9915132, and rs4006399). Second, ambiguous SNPs with non-concordant alleles or palindromic SNPs with ambiguous strand were removed (Wu et al., 2020). In the analysis of insomnia-LBP, short sleep duration-LBP, long sleep duration-LBP, and daytime sleepiness-LBP, rs10280045, and rs2644128; rs9474974; rs2973993; rs3803763, rs58460356, rs61696052, rs6557066, rs72831782, and rs9475029, respectively, were eliminated. Third, genetic variants associated with the outcome and confounders were eliminated using PhenoScanner. Only rs73196898 was excluded in the MR analysis of long sleep duration and LBP in the present study.

## Mendelian randomization analysis

Different methods were used to evaluate the causal relationship between five sleep characteristics and LBP. To further investigate the effect estimation of LBP on sleep traits, reverse casual inference was also implemented, and **Table 1** and **Supplementary Table 4** show the MR analysis results in detail. **Table 2** shows the details of GWAS summary statistics of LBP and sleep traits.

## Insomnia and low back pain

The causal effect of insomnia on LBP was the focus in the present study. The results showed a causal relationship between insomnia and LBP (IVW: OR = 1.954, 95% CI: 1.119–3.411, $p = 0.019$; MR-Egger: OR = 6.269, 95% CI: 1.388–28.313, $p = 0.025$; weighted median: OR = 2.095, 95% CI: 1.024–4.286, $p = 0.043$; maximum likelihood: OR = 1.998, 95% CI: 1.262–3.166, $p = 0.003$; penalized weighted median: OR = 1.958, 95% CI; 0.947–4.046, $p = 0.070$). Weak IVs due to the F statistics value was a low possibility ($F = 13.928$). The results of reverse causal inference indicated LBP can also have a causal effect on insomnia (IVW: OR = 1.015, 95% CI: 1.004–1.026, $p = 0.006$; MR-Egger: OR = 1.013, 95% CI: 0.988–1.039, $p = 0.317$; weighted median: OR = 1.012, 95% CI: 0.996–1.028, $p = 0.153$; maximum likelihood: OR = 1.016, 95% CI: 1.004–1.027, $p = 0.007$; penalized weighted median: OR = 1.012, 95% CI: 0.995–1.029, $p = 0.162$). The results of heterogeneity test are shown in **Table 1** and **Supplementary Table 5** and the output of heterogeneity analysis of insomnia-LBP indicated possible heterogeneity (MR-Egger: $Q$ statistics = 36.01452, $p = 0.07137978$; IVW: $Q$ statistics = 39.81181, $p = 0.04070194$). However, the reverse causal inference result appeared stable (MR Egger: $Q$ statistics = 25.74813, $p = 0.105658$; IVW: $Q$ statistics = 25.77981, $p = 0.1364423$). MR-Egger intercept

TABLE 1 Bidirectional MR analysis of casual effects between sleep traits and low back pain.

| Exposure | Outcome | nSNP | IVW | | | | | MR-Egger | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | OR(beta) | 95% CI | P value | Q statistics | P value | OR(beta) | 95% CI | P value | Q statistics | P value | intercept | P value |
| Insomnia | Low back pain | 27 | 1.954 | 1.119–3.411 | 0.0185 | 39.81181 | 0.0407019 | 6.269 | 1.388–28.313 | 0.025 | 36.01452 | 0.0713798 | −0.0161066 | 0.1170137 |
| Sleep duration | Low back pain | 4 | 1.44 | 0.851–2.436 | 0.1745 | 2.340923 | 0.5047265 | 0.449 | 0.038–5.263 | 0.589 | 1.439539 | 0.4868645 | 0.0354446 | 0.4426188 |
| Short sleep duration | Low back pain | 10 | 1.45 | 0.575–3.656 | 0.4309 | 12.09611 | 0.2079454 | 3.071 | 0.303–31.08 | 0.370 | 11.35524 | 0.1823715 | −0.0110508 | 0.4905873 |
| Long sleep duration | Low back pain | 19 | 1.566 | 0.887–2.765 | 0.122 | 28.88768 | 0.0497692 | 1.94 | 0.978–3.848 | 0.075 | 27.01214 | 0.0578901 | −0.0088114 | 0.2924465 |
| Daytime sleepiness | Low back pain | 29 | 0.423 | 0.153–1.166 | 0.096 | 41.70184 | 0.0462439 | 0.116 | 0.0002–55.157 | 0.499 | 41.43407 | 0.0373934 | 0.0087731 | 0.6794595 |
| Low back pain | Insomnia | 20 | 1.015 | 1.004–1.026 | 0.006 | 25.77981 | 0.1364423 | 1.013 | 0.988–1.039 | 0.317 | 25.74813 | 0.105658 | 0.0001958 | 0.883349 |
| Low back pain | Sleep duration | 20 | − 0.017 | −0.041–0.008 | 0.184 | 12.27915 | 0.873349 | 0.002 | −0.046–0.05 | 0.925 | 11.47538 | 0.8731067 | −0.0022579 | 0.3818029 |
| Low back pain | Short sleep duration | 20 | 1.004 | 0.993–1.016 | 0.443 | 12.61001 | 0.8579237 | 1.001 | 0.979–1.023 | 0.957 | 12.45992 | 0.822607 | 0.0004452 | 0.7029869 |
| Low back pain | Long sleep duration | 20 | 0.997 | 0.989–1.006 | 0.545 | 27.79357 | 0.0874826 | 0.998 | 0.978–1.019 | 0.870 | 27.77995 | 0.0654837 | −0.0001024 | 0.9261895 |
| Low back pain | Daytime sleepiness | 20 | 1.011 | 1.004–1.017 | 0.001 | 20.70746 | 0.3531877 | 1.002 | 0.989–1.014 | 0.786 | 18.16353 | 0.4449299 | 0.0010332 | 0.1297471 |

| Exposure | Outcome | nSNP | Weighted median | | | Maximum likelihood | | | Penalized weighted median | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | OR(beta) | 95% CI | P value | OR(beta) | 95% CI | P value | OR(beta) | 95% CI | P value |
| Insomnia | Low back pain | 27 | 2.095 | 1.024–4.286 | 0.043 | 1.998 | 1.262–3.166 | 0.003 | 1.958 | 0.947–4.046 | 0.070 |
| Sleep duration | Low back pain | 4 | 1.48 | 0.785–2.791 | 0.226 | 1.446 | 0.85–2.46 | 0.174 | 1.48 | 0.794–2.757 | 0.217 |
| Short sleep duration | Low back pain | 10 | 1.472 | 0.355–6.094 | 0.594 | 1.472 | 0.571–3.794 | 0.423 | 1.472 | 0.382–5.671 | 0.574 |
| Long sleep duration | Low back pain | 19 | 1.548 | 0.879–2.727 | 0.130 | 1.59 | 0.983–2.571 | 0.059 | 1.557 | 0.859–2.823 | 0.145 |
| Daytime sleepiness | Low back pain | 29 | 0.736 | 0.214–2.532 | 0.627 | 0.411 | 0.176–0.961 | 0.040 | 0.804 | 0.226–2.857 | 0.736 |
| Low back pain | Insomnia | 20 | 1.012 | 0.996–1.028 | 0.153 | 1.016 | 1.004–1.027 | 0.007 | 1.012 | 0.995–1.029 | 0.162 |
| Low back pain | Sleep duration | 20 | − 0.028 | −0.063–0.007 | 0.122 | − 0.017 | −0.042–0.008 | 0.184 | − 0.028 | −0.061–0.005 | 0.101 |
| Low back pain | Short sleep duration | 20 | 1.006 | 0.99–1.022 | 0.451 | 1.006 | 0.991–1.022 | 0.446 | 1.006 | 0.991–1.022 | 0.446 |
| Low back pain | Long sleep duration | 20 | 0.997 | 0.985–1.01 | 0.694 | 0.997 | 0.988–1.006 | 0.520 | 0.996 | 0.983–1.009 | 0.532 |
| Low back pain | Daytime sleepiness | 20 | 1.005 | 0.996—1.015 | 0.261 | 1.011 | 1.004–1.017 | 0.001 | 1.005 | 0.996–1.015 | 0.250 |

nSNP, number of single nucleotide polymorphism; IVW, inverse-variance weighted; OR, odds ratio; 95% CI, 95% confidence interval.



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TABLE 2 Description of six GWAS summary statistics.

| Phenotype | Ancestry | Sample size | nSNP | Consortium | Data sources |
|---|---|---|---|---|---|
| Insomnia | European | 336,965 participants | 30 | Neale Lab | ukb-a-13 (IEU OpenGWAS project) |
| Sleep duration | European | 128,266 participants | 6 | UK Biobank | PMID: 27494321 |
| Short sleep duration | European | 28,980 cases and 81,204 controls | 15 | NA | PMID: 27494321 |
| Long sleep duration | European | 10,102 cases and 81,204 controls | 24 | NA | PMID: 27494321 |
| Daytime sleepiness | European | 104,786 cases and 347,285 controls | 35 | UK Biobank | PMID: 31409809 |
| Low back pain | European | 13,178 cases and 164,682 controls | 20 | FinnGen | finn-b-M13_LOWBACKPAIN (IEU OpenGWAS project) |

nSNP, the number of single nucleotide polymorphism; PMID, ID of publication in the PubMed.

was used to evaluate the horizontal pleiotropy and the results are shown in Table 1 and Supplementary Table 6 (intercept $_{(insomnia-LBP)}$ = −0.01610656, $p_{(insomnia-LBP)}$ = 0.11 70137; intercept$_{(LBP-insomnia)}$ = −0.01610656, $p_{(LBP-insomnia)}$ = 0.1170137). MR-PRESSO analysis showed no obvious outliers.

## Sleep duration and low back pain

A causal relationship between sleep duration and LBP was not found (IVW: OR = 1.44, 95% CI: 0.851–2.436, $p$ = 0.175; MR-Egger: OR = 0.449, 95% CI: 0.038–5.263, $p$ = 0.589; weighted median: OR = 1.48, 95% CI: 0.785–2.791, $p$ = 0.226; maximum likelihood: OR = 1.446, 95% CI: 0.85–2.46, $p$ = 0.174; penalized weighted median: OR = 1.48, 95% CI: 0.794–2.757, $p$ = 0.217). A similar result was observed in reverse MR analysis (Supplementary Table 4). Abnormality was not found in the MR-PRESSO analysis, heterogeneity test, or MR-Egger intercept (Supplementary Tables 5–7).

## Short sleep duration and low back pain

The IVW analysis showed that genetic predisposition to short sleep duration did not increase the risk of LBP (OR = 1.45, 95% CI: 0.575–3.656, $p$ = 0.431) and a similar output was found in reverse MR analysis (OR = 1.004, 95% CI: 0.993–1.016, $p$ = 0.443). Heterogeneity test results and MR-PRESSO analysis in bidirectional causal inference were stable (Supplementary Tables 5, 7) and terms of intercept were −0.01105083 and 0.0004451583, respectively (Supplementary Table 6).

## Long sleep duration and low back pain

When estimating the causal effect of long sleep duration on LBP, results indicated the heterogeneity cannot be ignored (MR-Egger: $Q$ statistics = 27.01214, $p$ = 0.05789008; IVW: $Q$ statistics = 28.88768, $p$ = 0.04976916; Supplementary Table 5). Therefore, the output of random effect IVW analysis was considered to be more reliable. Next, the genetic predisposition to long sleep duration was shown to not affect the risk of LBP (OR = 1.566, 95% CI: 0.887–2.765, $p$ = 0.122) and the results of reverse causal inference were similar (OR = 0.997, 95% CI: 0.989–1.006, $p$ = 0.545) with terms of intercept −0.008811378 and −0.000102443, respectively (Supplementary Table 6). MR-PRESSO analysis showed no significant outliers (Supplementary Table 7).

## Daytime sleepiness and low back pain

The results of the sensitivity analysis are summarized in Supplementary Tables 5, 6; both MR-Egger and IVW analysis showed heterogeneity (MR-Egger: $Q$ statistics = 41.43407, $p$ = 0.03739338; IVW: $Q$ statistics = 41.70184, $p$ = 0.04624392) and random effect IVW analysis showed no causal relationship between daytime sleepiness and LBP. However, the MR-PRESSO analysis identified an outlier (rs4765936). When comparing the causal effect before and after removing this outlier, bias was not found in the adjusted result (raw: causal estimate = −0.3669115, $SE$ = 0.493133, $T$-stat = −0.7440417, $p$ = 0.4619627; outlier-corrected: causal estimate = −0.1740137, $SE$ = 0.4572936, $T$-stat = −0.3805295, $p$ = 0.705991; Supplementary Table 7). Reverse causal inference indicated that genetic predisposition to LBP could affect the risk of daytime sleepiness (IVW: OR = 1.011, 95% CI: 1.004–1.017, $p$ = 0.00114; maximum likelihood: OR = 1.011, 95% CI: 1.004–1.017, $p$ = 0.00144). MR-Egger intercept did not show horizontal pleiotropy [Intercept $_{(daytimesleepiness-LBP)}$ = 0.008773135; Intercept $_{(LBP-daytimesleepiness)}$ = 0.001033245]. Outliers were not identified in MR-PRESSO analysis and more detailed results are shown in Supplementary Tables 5–7.

## Visualization

Leave-one-out analysis and funnel plot results are shown in Supplementary Figures 1, 2. Due to the existence of individual, potentially influential SNP, the results should be interpreted with caution. The funnel plot showed no probable directional pleiotropy. Figure 1 shows the individual putative causal effect between insomnia and LBP. The intercepts calculated in every method of MR analysis were close to zero which indicated

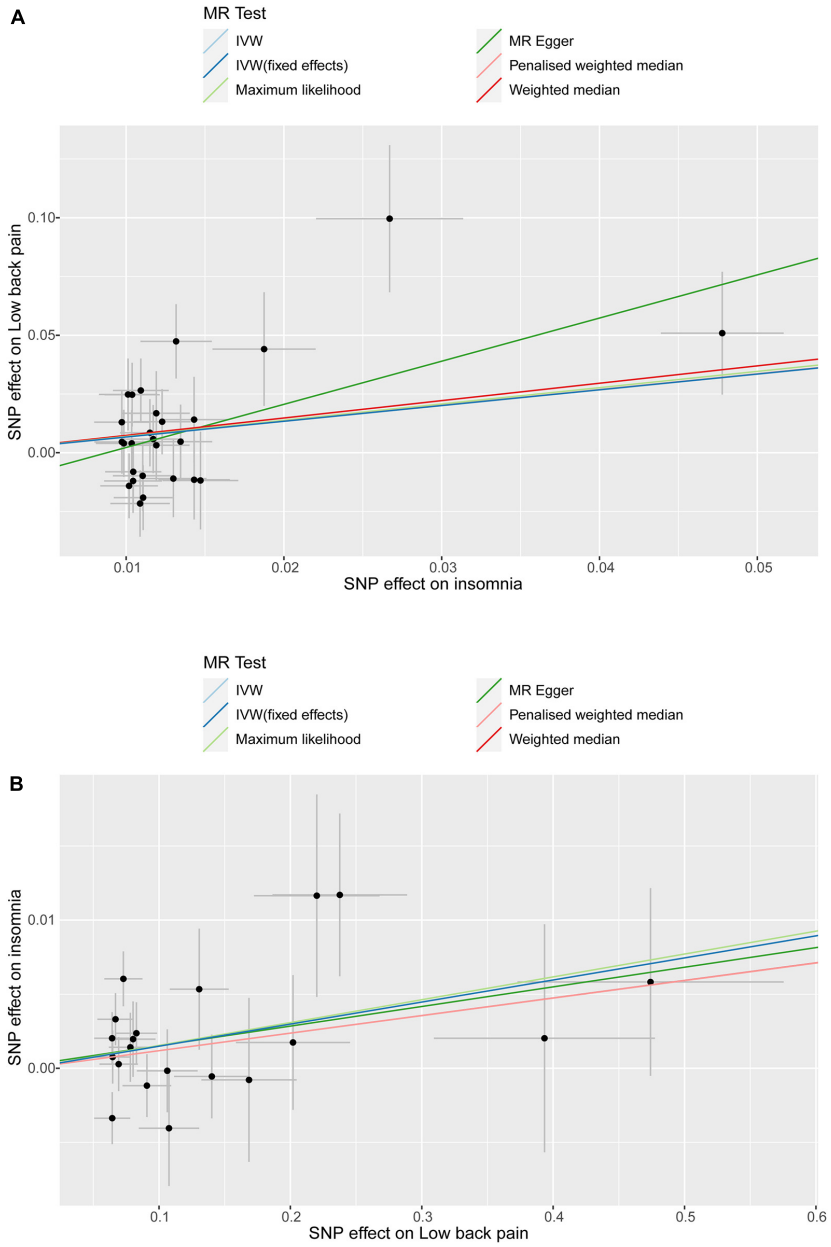


FIGURE 1
Individual estimates about the potential causal effect of insomnia and low back pain. The *x*-axis shows that the single nucleotide polymorphism (SNP) effect on insomnia in **(A)** and the *y*-axis shows the SNP effect on low back pain; **(B)** shows the results of the reverse causal inference. Several methods of MR analysis including MR Egger, weighted median, inverse variance weighted (IVW), maximum likelihood(ML) and penalized weighted median were performed.

the possibility of horizontal pleiotropy was low. A significant positive correlation was observed between insomnia and LBP. Figure 2 shows the causal effect estimates between each SNP and the outcome, and the combination of the effect estimates based on IVW and MR-Egger regression. Supplementary Table 3 shows the $p_{exposure}$ of every SNP was less than $p_{outcome}$, thus, additional SNPs were not removed in this procedure. Figure 3 shows the design flow chart for the MR study.

## Discussion

The study results supported our hypothesis. Specifically, MR analysis showed that insomnia significantly increases the risk of LBP (IVW: OR = 1.954, 95% CI: 1.119–3.411, $p$ = 0.019). Based on the possible heterogeneity, the results were referred to the random effects IVW. Except the outcome assessed using penalized weighted median (OR = 1.958, 95%

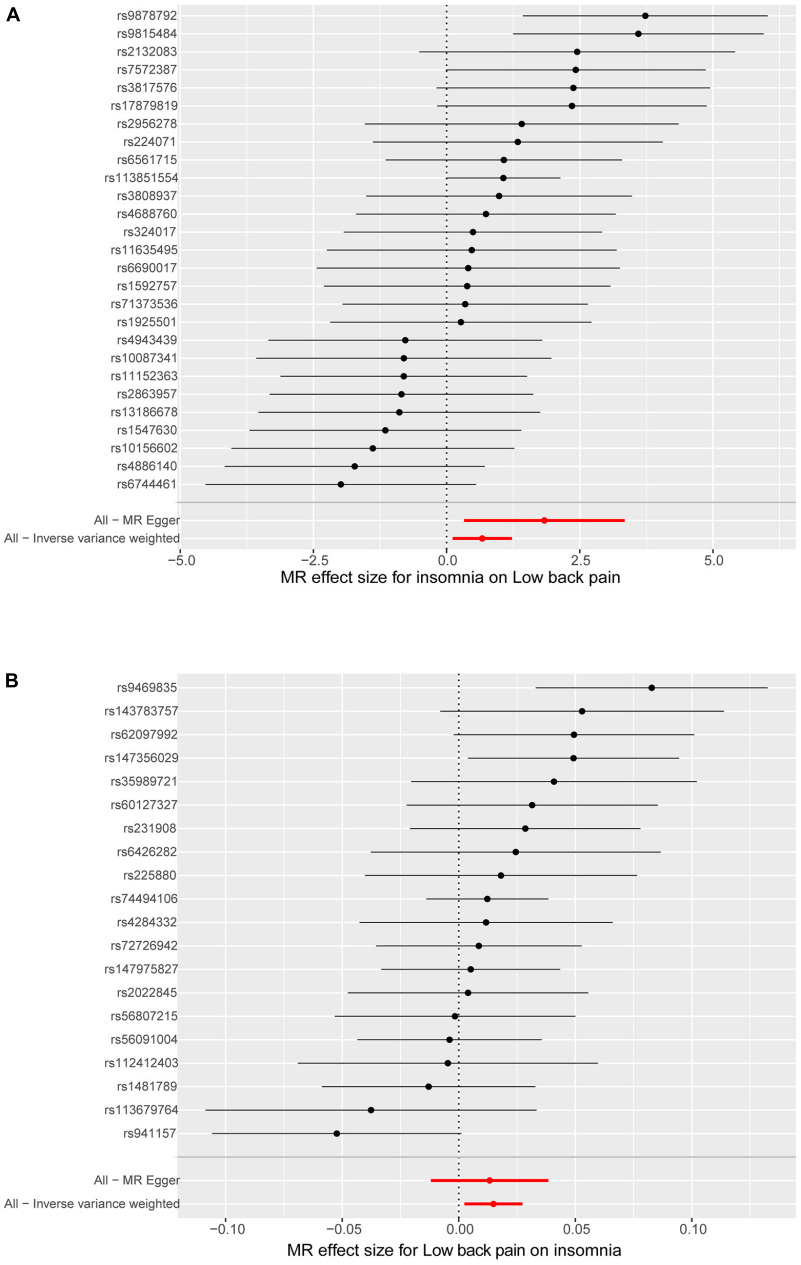


FIGURE 2
Forest plot of the causal effects between sleep disturbance and low back pain. In **(A)**, the *x*-axis shows that the MR effect size for insomnia on low back pain, and the *y*-axis shows the effect for each of the single nucleotide polymorphisms (SNPs); **(B)** shows the results of the reverse causal inference. MR Egger and inverse variance weighted (IVW) analyze the total effect of the genetic variants (or exposure) on the outcome.

CI: 0.947–4.046, $p = 0.070$), the results of other MR analysis methods showed good consistency. All the ORs calculated using different methods were > 1, indicating the results of five MR analysis methods showed that insomnia patients increased the risk of LBP. The reverse MR analysis showed LBP also increased the risk of insomnia (IVW: OR = 1.015, 95% CI: 1.004–1.026, $p = 0.006$). Horizontal pleiotropy and outliers were not identified in sensitivity analysis, confirming the stability of results.

In previous observational studies, the potential association between sleep disturbance and LBP were reported. Axen and colleagues reported sleep disturbance in roughly 67% of the LBP patients included in a prospective study (Axen, 2016). This result reveals the possible coexistence between sleep disturbance and LBP. In a 3-year longitudinal study, a relationship was observed between sleep disturbance and LBP, and the duration and frequency of sleep disturbance were significantly associated with the development of LBP, becoming stronger as the two

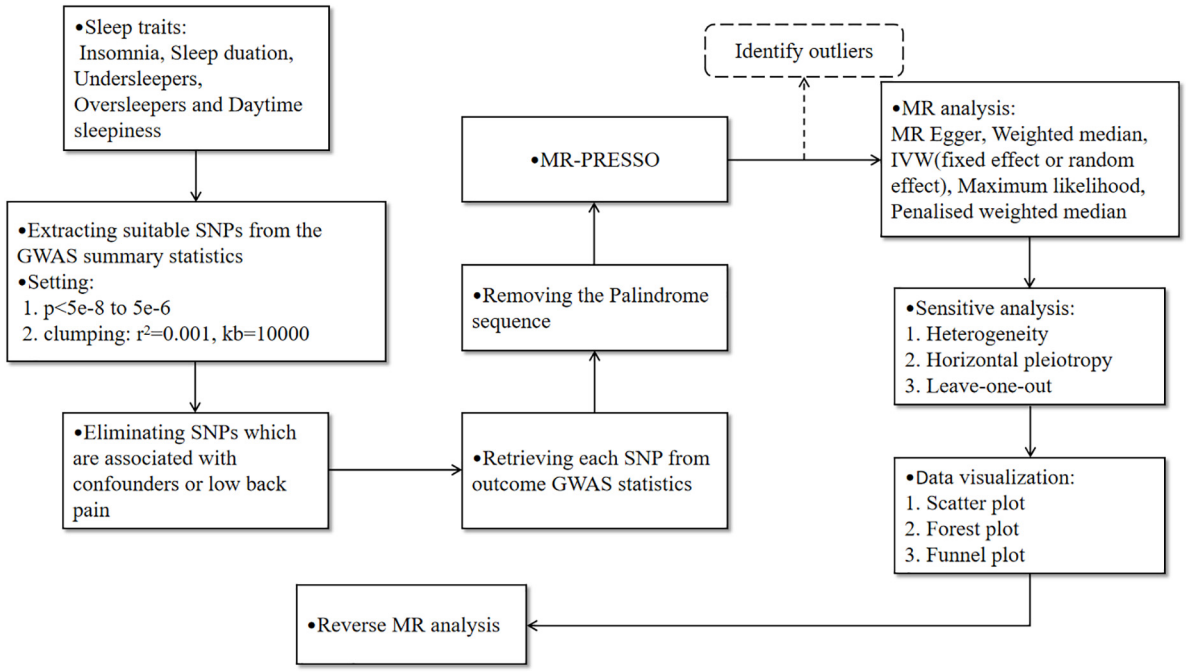


FIGURE 3
The design flow chart for the Mendelian randomization (MR) study. The dotted line indicates that the outliers may be eliminated after identification by MR-PRESSO, which needs to be judged based on the stability of the results before and after adjustment.

variables increased (Yabe et al., 2022). In addition, an interaction was found between LBP and sleep disturbance in which chronic daytime pain reduced the quality of nighttime sleep, subsequently causing worse pain the next day and further contributed to sleep disturbance (Kelly et al., 2011). Due to the growing amount of evidence from different research, strictly controlling for potential confounders is difficult, and a bidirectional association between sleep disturbance and LBP may exist (Finan et al., 2013; Alsaadi et al., 2014). Genetic variants and allelic randomization minimize the problem of confounding issues and reverse causation, providing stronger evidence than traditional observational studies in inferring cause-effect relationships (Davies et al., 2018).

The results of our two sample MR analysis supported the general theory of most observational research (Bahouq et al., 2013; Skarpsno et al., 2020). Sleep disturbances, such as insomnia, can significantly increase the risk of LBP, and the patients who experienced LBP were more likely to develop insomnia.

Previous MR studies have also showed the similar results. Sun et al. (2020) reported a causal relationship between sleep disturbance and chronic pain. Researchers replaced sleep disturbance with insomnia (the most common sleep disturbance) because they could not find GWAS summary statistics for sleep disturbance. Their study not only revealed the causal relationship between insomnia and chronic pain, but also provided the guidance for further research. In the present study, we limited the scope of the outcome, because LBP was the outcome that we were interested in. The inclusion of different sleep traits also helps us to explore the relationship between phenotypes other than insomnia and outcomes.

Debate remains regarding the specific mechanisms by which sleep deprivation affects pain. Nitric oxide (NO), considered a key element in the control of sleep and wake homeostasis, may have a significant role in both pain regulation and sleep. An increase of NO in rats with sleep deprivation was previously demonstrated, and that basal forebrain NO increases during sleep deprivation begin before frontal cortex increases in iNOS and NO. Other evidence showed application of a neuronal nitric oxide synthase (nNOS) inhibitor can attenuate mechanical hypersensitivity, indicating the increase of NO promotes hyperalgesia, and in the rat model of chronic pain, can worsen the pain inhibition activity in periaqueductal gray (PAG) area after sleep deprivation, resulting in severe pain (Kalinchuk et al., 2006; Smith et al., 2007; Tomim et al., 2016; Haack et al., 2020). Another study (Devine et al., 2019) reported that increased basal cortisol level and hyperreactivity of the Hypothalamus-pituitary-adrenal (HPA) axis to stressors were found in people who underwent insomnia. It was gratifying that this hyper-reactivity has been proved to involve in the relationship between insomnia and mechanical hypersensitivity (Goodin et al., 2012). Prostaglandin (PG), a classic inflammatory marker, was proved to mediate inflammatory pain. In an animal study, researchers found that the levels of PGs were significantly

increased in the cerebrospinal fluid (CSF) of rats (Ram et al., 1997). Therefore, this potential mechanism may be complex and diverse.

On the other hand, we prefer that the results of MR analysis can provide guidance for the clinical treatment of LBP. For patients with chronic LBP, pain management and enhanced quality of life remains a significant and meaningful issue. Taking analgesic medications is an important method to manage chronic LBP (Koes et al., 2010; Shaheed et al., 2016), although frequent use to alleviate symptoms has less desirable therapeutic effects. Some evidence has shown that if LBP persists for longer than 12 weeks, physicians should concentrate more on pain management and enhancing quality of life rather than focusing on pain resolution (Golob and Wipf, 2014). For patients with chronic pain, sleep disturbance is an important factor that can reduce the quality of life.

Recently, the treatment of chronic pain was suggested to be impaired by poor and untimely intervention for insomnia (Nijs et al., 2018). Conversely, adding sleep regulator actively to pain-targeted therapy for patients suffering from insomnia may produce positive and unexpected effects. This assumption was supported by other research. In a double-blind trial, the researchers found significant improvement in sleep quality and pain grade in the observation group after adding eszopiclone to a standard naproxen pain relief regimen (Goforth et al., 2014). However, the long-term use of benzodiazepines should be taken into consideration due to the numerous adverse effects.

Melatonin (MT), a neurohormone that is mainly synthesized and secreted within the pineal gland, plays a significant role in humans as a powerful circadian regulator (Pfeffer et al., 2018). Externally applied melatonin can be used to treat the desynchronization of circadian rhythms that coursed by various factors. As a classic drug for the treatment of sleep disorders, melatonin has been used for improving sleep in patients with insomnia mainly because it does not cause hangover or show any addictive potential (Cardinali et al., 2012). In another study, researchers compared the efficacy in the observation group (3 mg melatonin 30–40 min before bedtime) and the control group (without melatonin) in patients with LBP for at least 12 weeks (Kurganova and Danilov, 2015). A significant reduction in pain intensity upon movement and in the resting state was observed in the observation group compared with the control group, which is in agreement with our MR results. Therefore, based on the above two studies, we speculated that the combination of sleep regulators and pain-directed therapy in integrated pain management of LBP patients may produce positive outcomes. However, the potential mechanism still needs more research to confirm.

As mentioned above, the results of MR analysis indicate a bidirectional causal relationship exists between insomnia and LBP. The treatment of LBP requires diversified and integrated management objectives, thus, other pain-related issues also should be considered in the treatment objectives for comprehensive management. Improvement of sleep disturbance is beneficial for improving the quality of life in patients with LBP, and use of sleep regulators may aid in achieving the goal of comprehensive pain management. But this hypothesis needs to be supported by more high-quality cohort studies with large samples or randomized controlled trials (RCTs).

## Limitations

The present study had several limitations. First, the participants included in this study were of European ancestry, thus, the results may not be equally applicable to other ethnic groups with different cultural backgrounds, geographical environments, living habits, and other factors. Second, several methods were used to identify and evaluate abnormal genetic variants, however, the possible effect of intermediation or pleiotropy on the results cannot be completely excluded. Then, sleep disturbance captures a much wider range of sleep concerns than just these characteristics, so more sleep traits may be considered in the MR analysis. Finally, it may be better to calculate the estimations within the subgroup based on gender stratification.

## Conclusion

A causal relationship was found between insomnia and LBP, and sensitivity analysis results tended to be robust. Sleep regulators may need to be considered in the comprehensive management of LBP with more evidences of perspective studies.

## Data availability statement

All the data used in this study came from the publicly available datasets. Related raw data can be visited and obtained in https://www.kp4cd.org/dataset_downloads/sleep and https://gwas.mrcieu.ac.uk/datasets/.

## Ethics statement

This study used the published articles or publicly available GWAS summary data. We did not collect additional raw data, and therefore approval from medical ethical committee is not required. Each study included has been approved by their institutional ethics review committees.

## Author contributions

GL, JT, TW, and YY collected and analyzed the data. YY and MY revised and reviewed the final approval of the manuscript. All authors revised and reviewed the manuscript.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2022.1074605/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Leave-one-out analysis.

SUPPLEMENTARY FIGURE 2
Funnel plot.

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# Sleep deprivation and NLRP3 inflammasome: Is there a causal relationship?

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In the modern era, sleep deprivation (SD) is one of the most common health problems that has a profound influence on an individual's quality of life and overall health. Studies have identified the possibility that lack of sleep can stimulate inflammatory responses. NLRP3 inflammasome, a key component of the innate immune responses, initiates inflammatory responses by enhancing proinflammatory cytokine release and caspase-1-mediated pyroptosis. In this study, NLRP3 modification, its proinflammatory role, and potential targeted therapies were reviewed with regard to SD-induced outcomes. A growing body of evidence has showed the importance of the mechanistic connections between NLRP3 and the detrimental consequences of SD, but there is a need for more clinically relevant data. In animal research, (i) some animals show differential vulnerability to the effects of SD compared to humans. (ii) Additionally, the effects of sleep differ depending on the SD technique employed and the length of SD. Moreover, paying attention to the crosstalk of all the driving factors of NLRP3 inflammasome activation such as inflammatory responses, autonomic control, oxidative stress, and endothelial function is highly recommended. In conclusion, targeting NLRP3 inflammasome or its downstream pathways for therapy could be complicated due to the reciprocal and complex relationship of SD with NLRP3 inflammasome activation. However, additional research is required to support such a causal claim.

KEYWORDS

**sleep, sleep deprivation, inflammation, inflammasome, neuroinflammation**

## Introduction

Sleep is a universal phenomenon of life and is critical for survival. It can be termed as a spontaneously reversible state of body and mind, distinguished by muted consciousness and lowered behavioral reactivity to external stimuli. It has also been shown that it plays important roles in almost every aspect of our health and wellbeing. Adequate sleep is necessary for proper functioning of the body, and inadequate sleep has important health consequences including cardiovascular diseases, neurological disorders, and even early mortality, which comprise a substantial portion of healthcare costs worldwide (Chattu et al., 2019).

Inflammation is an important innate immune response to potentially dangerous substances including pathogens, chemicals, and cell debris. Inflammatory responses play a critical role in protecting the body by detecting and removing injurious stimuli. Inflammation is intended to be beneficial to healthy tissues, but when it happens persistently, it will be detrimental. It is strongly related to various disorders including rheumatoid arthritis, hypertension, diabetes, neurological diseases, various infections, and some types of cancer (Amini et al., 2020b; Yamamoto et al., 2021; Yousefi et al., 2021, 2022).

Sleep deprivation (SD) is considered to be a result of lifestyle habits, neurological problems, and different sleep disorders such as insomnia and obstructive sleep apnea (OSA) (Amini et al., 2020a; Hanson and Huecker, 2021). SD, meaning insufficient and irregular sleep, has been associated with induction of inflammation in the entire body (Wang et al., 2021). Emerging evidence suggests that inflammation plays a key role in progression of SD-related impairments that are often not easily recognized. Thus, projects funded under this topic are highly encouraged. Despite decades of research, the mechanisms of the effects of SD on inflammation in the body have remained a mystery. Generally, insufficient sleep induces the release of important inflammatory cytokines such as pro-inflammatory cytokines tumor necrosis factor (TNF)-alpha, interleukin (IL)-1β, and IL-6 (Chennaoui et al., 2015). However, there are no specific inflammatory mediators that are involved in the response to sleep manipulation and its related mechanisms. This may partly be owing to the fact that SD-induced consequences are mostly reported in the central nervous system or are a result of some pre-existing inflammatory conditions such as obesity or type 2 diabetes mellitus. According to the results of recent investigations, inflammasomes can act as primary activators of inflammatory cytokines, thereby inducing inflammation. Inflammasomes are multimeric complexes of receptors and sensors of innate immune responses that through various mechanisms, play a critical role in inducing inflammatory responses against different pathogens (Noori et al., 2021). Although the actual role of inflammasomes in maintenance of immunologic homeostasis has not been clearly identified, they play an important role in clearing pathogens and damaged cells under physiological conditions. Whereas under pathological conditions, their overactivation may lead to disease onset or development (Höchsmann et al., 2019; Zhang H. et al., 2021). Nod-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome is the most well-documented inflammasome type. The aberrant expression of NLRP3 inflammasome plays an important role in a wide range of disorders (Jourdan et al., 2013; Fusco et al., 2020). Recently, an increasing amount of data has demonstrated that activation of NLRP3 inflammasome is a critical mechanism in sleep modulation (Zielinski et al., 2017). In this review, light will be shed on the recent progress made in understanding the activation of NLRP3 inflammasomes and its potential therapeutic role in SD-induced inflammation, as it may be effective in treatment of a broad range of SD-induced health effects.

## Neuronal mechanisms of sleep

Sleep is a common physiological behavior in almost all creatures and it accounts for approximately one-third of a human's life, and lack of sleep has major clinical consequences. There are two forms of sleep: rapid eye movement (REM) sleep and non-REM (NREM) sleep (which has three stages). NREM and REM types of sleep alternate repeatedly during the night (Amini et al., 2020a). Different brain-wide neural networks are active during these two forms of sleep. Sleep normally starts with a NREM sleep stage, progresses through three NREM periods, and continues with a REM period. There are several NREM-promoting centers and they act as follows: The ventrolateral preoptic area (VLPO) GABAergic neurons, by inhibiting wake-promoting neurons in the hypothalamus and brainstem, the basal forebrain areas *via* ascending projections toward the cortex, the GABAergic parafacial zone by inhibiting the parabrachial nucleus, and the scattered cortical sleep-active neurons that contain both GABA and neuronal nitric oxide synthase. The neural networks in the pons are required for induction of REM sleep. Glutamatergic neurons in the sublaterodorsal nucleus (SLD) stimulate spinal inhibitory interneurons (GABAergic/glycinergic neurons) in the ventromedial medulla and spinal cord, resulting in motor neuron hyperpolarization and muscle tone reduction. Cholinergic pedunculopontine (PPT) and laterodorsal tegmental nucleus (LDTg) neurons also induce REM sleep by sending projections to the thalamus, whereas aminergic neurons block them during awakening and NREM sleep (España, 2017; Scammell et al., 2017; **Figure 1**).

## Circadian regulation of sleep

A biological clock is an internal mechanism that synchronizes the timing of internal rhythms with external light.

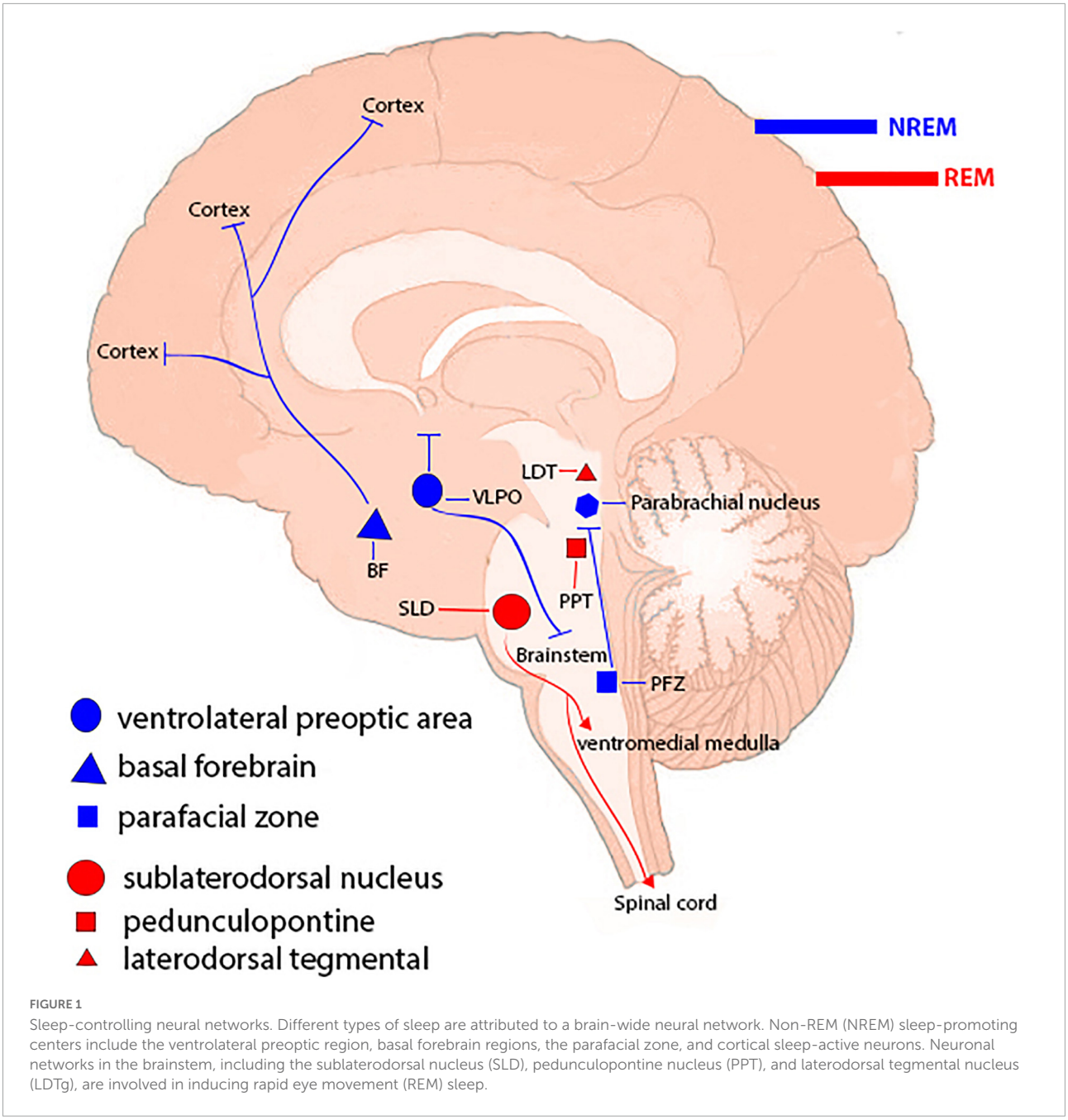


FIGURE 1
Sleep-controlling neural networks. Different types of sleep are attributed to a brain-wide neural network. Non-REM (NREM) sleep-promoting centers include the ventrolateral preoptic region, basal forebrain regions, the parafacial zone, and cortical sleep-active neurons. Neuronal networks in the brainstem, including the sublaterodorsal nucleus (SLD), pedunculopontine nucleus (PPT), and laterodorsal tegmental nucleus (LDTg), are involved in inducing rapid eye movement (REM) sleep.

The hypothalamus regulates it through the suprachiasmatic nucleus, which gets sensory input from the retinohypothalamic tract (RHT) based on luminance detected by the retina. Small projections from the RHT may also have an effect on the activity of sleep-promoting neurons in the preoptic region. Sleep pressure (extended intervals of alertness) drives the body to sleep when a certain amount of time has passed and regulates sleep intensity. NREM sleep-promoting substances (somnogens) such as adenosine (AD), prostaglandin D2, and cytokines such as IL-1 and TNF-α are most likely involved in this sleep/wake homeostatic response. The interplay of the circadian rhythm and sleep homeostasis can regulate timing, depth, and duration of sleep. In fact, the alignment between these two processes is crucial for the construction, maintenance, and function of the body and the brain (Deboer, 2018; Christensen et al., 2019). In addition, SD has been associated with many physiological and psychological disorders. For example, in healthy individuals, SD has been linked to diabetes type 2 and obesity, increased risk of cardiovascular diseases, and impairment of the functions of the immune system (Antza et al., 2022).

## Sleep deprivation epidemiology

Sleep deficiency can occur due to various circumstances and a broad spectrum of pathophysiological reasons including medication, debilitating diseases, neurological disorders, and sleep disorders. It is not clear how much an adult needs sleep, but it is considered to be 7–9 h per day. The American Academy of Sleep Medicine (AASM) and the Sleep Research Society (SRS) have stated that adults sleep less than 8 h a night (Chattu et al., 2019). As it appears, over a third of the U.S. citizens do not get enough sleep, as revealed in the latest survey by the Centers for Disease Control and Prevention (CDC) (Chattu et al., 2019). The U.S. National Academy of Medicine estimates that hundreds of billions of dollars are spent each year caring for people with sleep disturbances. For instance, one fifth of all the damage caused by major car collisions is attributed to drowsy driving (Higgins et al., 2017; Tefft, 2018). However, lack of sleep is not a concern for only the United States; it also affects other developing as well as developed countries such as the United Kingdom, the Netherlands, and Canada. According to a comprehensive review of the available literature, factors such as age, gender, race/ethnicity, and climatic conditions are the most common determinants that affect the prevalence of SD (Patel et al., 2010; Grandner et al., 2016). A study in Saudi Arabia showed that sleep loss is quite common among adolescents, with a higher incidence on weekdays (46%) than weekends (33%) (Nasim et al., 2019). In another study conducted in four cities in China, the incidence of insomnia was recorded at 37.75%, and less than 11.1% of the individuals suffering from insomnia took sleep medications on a regular schedule (Wang et al., 2016). It has transpired that adolescents who do not achieve adequate sleep are tend to gain weight primarily because of physical inactivity, they may suffer from depressive symptoms, take part in dangerous practices (i.e., drinking, smoking, drug use, etc.), and struggle with school (Pasch et al., 2010; Chattu et al., 2018). Finally, SD is a serious health problem due to its overall deleterious effects on the body. Given the evident implications of SD, tools for preventing and controlling it are highly demanded.

## Prevention and control of sleep deprivation

Overlooking the long-term aggregated impact of sleep restriction can accelerate the development of metabolic syndrome, cardiovascular diseases, diabetes, stroke, etc. (García-Aviles et al., 2021). A broad range of non-pharmacological and pharmacological therapies are available for patients with SD. Non-pharmacological approaches generate the most valid, reliable, and sustainable clinical advantages with the lowest levels of cost and complications. The first-line and best option for treatment of insufficient sleep is prevention by different mechanisms including creating a consistent schedule, avoiding caffeine and alcohol, having a bedtime ritual, and unplugging all extraneous electrical equipment. Pushing back school start times and increasing public awareness of the importance of adequate sleep and the consequences of SD, as well as sleep hygiene education programs are some examples of dealing with this problem. However, pharmacological approaches should be introduced as an alternative treatment option to improve sleep in patients when non-pharmacological interventions are ineffective or have failed. The primary treatment plans of sleep pharmacotherapy improve waking functions by improving sleep or by increasing energy during wakefulness. Alertness-promoting substances include amphetamine derivatives, modafinil, and caffeine. The most commonly-reported sleep-promoting substances are melatonin and zolpidem (Proctor and Bianchi, 2012; MacLeod et al., 2018; Jameie et al., 2019; Brito et al., 2020). Using medications to initiate extremely long periods of wakefulness could promote deleterious health consequences. Besides, frequent and patterned use of the medications stated here may actually result in creation of various problems such as physical or psychological dependency (De Matos et al., 2017). In general, a consistent schedule is a major element in this process, as is the cautious application of pharmacological drugs that improve sleep by enhancing alertness or aiding sleep (MacLeod et al., 2018; Jameie et al., 2019).

## The NLRP3 inflammasome signaling pathway

Inflammasomes are the most important components of innate inflammatory responses to harmful irritants. Once activated, they cause a rapid and highly inducible proinflammatory response. There are four types of inflammasomes: absentinmelanoma2 (Aim2), nucleotide-binding domain leucine-rich repeat-containing receptor 1 (thepyrin domain-containing NLRP1), NLRP3, and Nod-like receptor CARD domain-containing 4 (NLRC4). Although different inflammasomes have been identified, NLRP3 is one of the most well-characterized inflammasomes (Bazrafkan et al., 2018). In this section, the mechanisms that lead to activation of NLRP3 inflammasome are introduced and discussed. NLRP3 is composed of nucleotide-binding oligomerization domain (NACHT), apoptosis-speck-like protein (ASC), and procaspase-1 protein. NLRP3 inflammasome is mainly activated by pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs). It has been revealed that two signals are involved in activation of NLRP3 inflammasome. The first signal (priming) is provided by different cytokines or engagement of various PAMPs and DAMPs with pattern recognition receptors (PRRs) that lead to activation of nuclear factor-κB (NF-κB) and transcriptional

upregulation of NLRP3 components, pro-IL-18, and pro-IL-1β. In addition, NF-κB activation minimizes the activation threshold of NLRP3 inflammasome through additional post-translational modification (PTMs) (Yang et al., 2019; Zhao and Zhao, 2020). The second signal (activation) is initiated by a wide range of stimulations that appear during tissue damage, infections, etc., resulting in NLRP3 activation and formation of an inflammasome complex. Formation of NLRP3 complex results in activation of caspase 1, which induces the secretion of the activated form of pro-IL-1β and pro-IL-18 cytokines and development of inflammatory responses. Release of these cytokines plays a critical role in initiation of the host defense pathways and eradication of various pathogens. In addition, activation of inflammasomes leads to induction of an inflammatory form of cell death called pyroptosis (Paik et al., 2021; **Figure 2**). Previous studies have revealed that multiple brain-based immune proteins may contribute in sleep regulation by means of production of cytokines, phagocytosis or response to pathogens, as well as mRNA expression of the components of the NLRP3 inflammasome. According to a growing body of research, NLRP3 inflammasome plays a critical role in spontaneous sleep and sleep responses following sleep loss (Pourcet and Duez, 2020). It seems that the activity of NLRP3 inflammasome is a possible mechanism in sleep-modulation as well as increased cerebral blood flow (CBF) following SD, particularly in NREM sleep, as demonstrated in the electroencephalographic slow-wave activity (EEG SWA) (Zielinski et al., 2017, 2020). An effective activation of NLRP3 inflammasome is required for emergence of appropriate innate immune responses against various pathogens and prevention of catching or spreading pathogens. However, activation of these immune system proteins should be precisely controlled to avoid hyperinflammation or excessive deleterious effects (Zhang Y. et al., 2021). Recently, various studies have revealed a positive link between perturbation of the circadian clock and inflammation (Zielinski et al., 2020; Paik et al., 2021). Therefore, the underlying mechanisms and the related consequences remain a point of concern and should be the center of interest. SD leads to neuroendocrine dysregulation and may activate NLRP3 inflammasome (**Figure 3**).

## Role of NLRP3 inflammasome in SD-induced neuroinflammation

The innate immune system that uses inflammatory responses plays an important role in protection against various pathogens. The function of inflammation is eliminating and/or controlling damaged agents or the injurious components of tissues *via* phagocytosis as well as clearing them by inflammasomes (Evavold and Kagan, 2019). Neuroinflammatory response is a complex inflammatory response within the central nervous system. This inflammatory response is mediated by different cells such as microglia and astrocytes. Activation of glial cells leads to a variety of functions, including phagocytosis and release of some inflammatory mediators including cytokines, chemokines, reactive oxygen species, and secondary messengers (Brusaferri et al., 2022). SD can impair the normal functions of the nervous system, particularly brain functions such as memory, learning, decision making, and attention. In addition, SD has adverse effects on the rest of the body. For example, it impairs certain functions of the immune system and increases susceptibility to different infections. Over the last decade, neuroinflammation has been considered as a putative pathophysiological mechanism that contributes to the detrimental effects of sleep disturbance (Green et al., 2020). Mitogen-activated protein kinase (MAPK) signaling pathway plays an important role in transduction of extracellular signals to cellular responses, and it has been identified as a novel regulator of NLRP3 inflammasome activation. The stimulation of MAPK/NLRP3 axis in the hippocampus CA1 area, which is reversed by sleep recovery, is proposed as one of the mechanisms involved in this process (Fan et al., 2021). However, SD has been suggested to be an oxidative challenge for the brain. SD-induced oxidative stress can activate the NLRP3 inflammasome in rat hippocampus (Smith et al., 2021). In addition, previous research has shown that MCC950, as a potent and specific inhibitor of the NLRP3 inflammasome, can reverse SD-induced neuroinflammation and microglia activation by targeting NLRP3 (Smith et al., 2019). However, the precise mechanism by which SD causes neuroinflammation in the brain is not yet fully elucidated. There are many challenging questions that remain to be addressed. For instance, we know that insufficient sleep stimulates the NLRP3 inflammasome in neurons, microglia, and astrocytes. However, different effects have been observed depending on the involved portion of the brain (Niznikiewicz et al., 2017). The effects of NLRP3 inflammasome have been reported in the hippocampal region, but its effects have not yet been explored in other brain areas and thus remain unknown.

## SD-induced cognitive decline: Targeting NLRP3 inflammasome

People subjected to both total and partial SD, generally suffer from cognitive performance deficits as well as mood swings. Modafinil is a widely-used stimulant that was originally developed to treat paroxysmal narcolepsy. According to different research studies, modafinil improves NLRs inflammasome-mediated pyroptosis in mice with SD through increasing BDNF (brain-derived neurotrophic factor) stimulation in the hippocampus and synaptic plasticity, which in turn, improve learning, memory, and cognitive functions (Xiong et al., 2022).

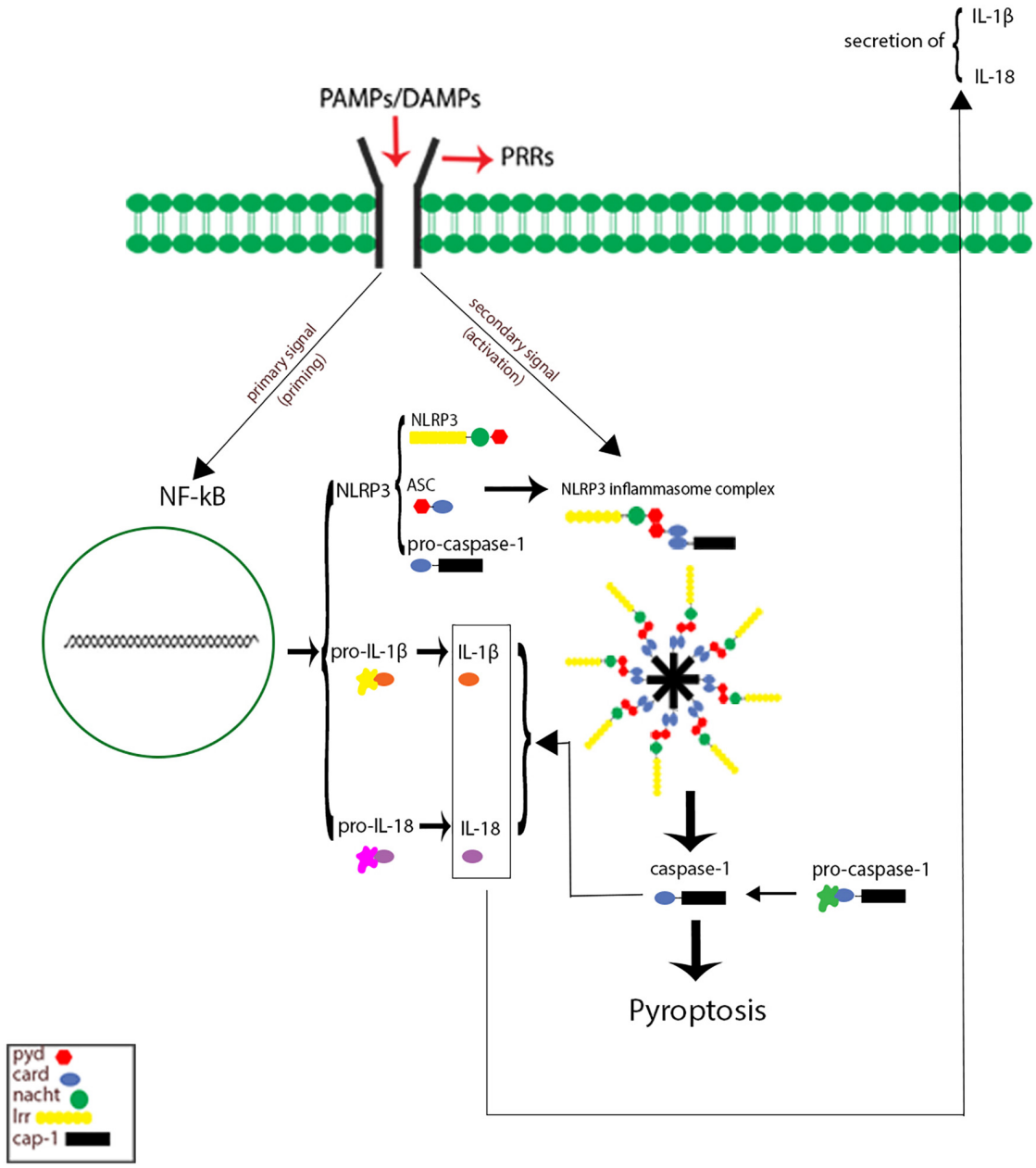


FIGURE 2
Signaling pathways of Nod-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome. The first signal (priming: left) initiated through engagement of pathogen-associated molecular pattern (PAMPs) and danger-associated molecular patterns (DAMPs) with different pattern recognition receptors (PRRs), which in turn leading to activation of nuclear factor κB (NF-κB) and gene transcriptional of NLRP3 components, and pro inflammatory cytokines pro-IL-1 and pro-IL-18. The secondary signal (activation: right) is provided by a wide range of PAMPs and DAMPs that leading to formation of NLRP3 inflammasome complex. This complex activates caspase 1 enzyme, which resulting cleaves and secretion of activate forms of pro-IL-1 and pro-IL-18 as well as inducing cell death pyroptosis.

## SD and depression: The role of NLRP3 inflammasome

The association between sleep and mental health disorders such as depression has been demonstrated through a number of research studies. Depression, as a common mood condition, is defined as feelings of unhappiness, loss, or rage that can interfere with daily activities and result in poor functional outcomes. It is believed that a total of 5% of adults experience depression. Poor sleep is both a potential risk and an indicator of depression. Studies have demonstrated that depression may be enhanced due to acute sleep deprivation or vice versa.

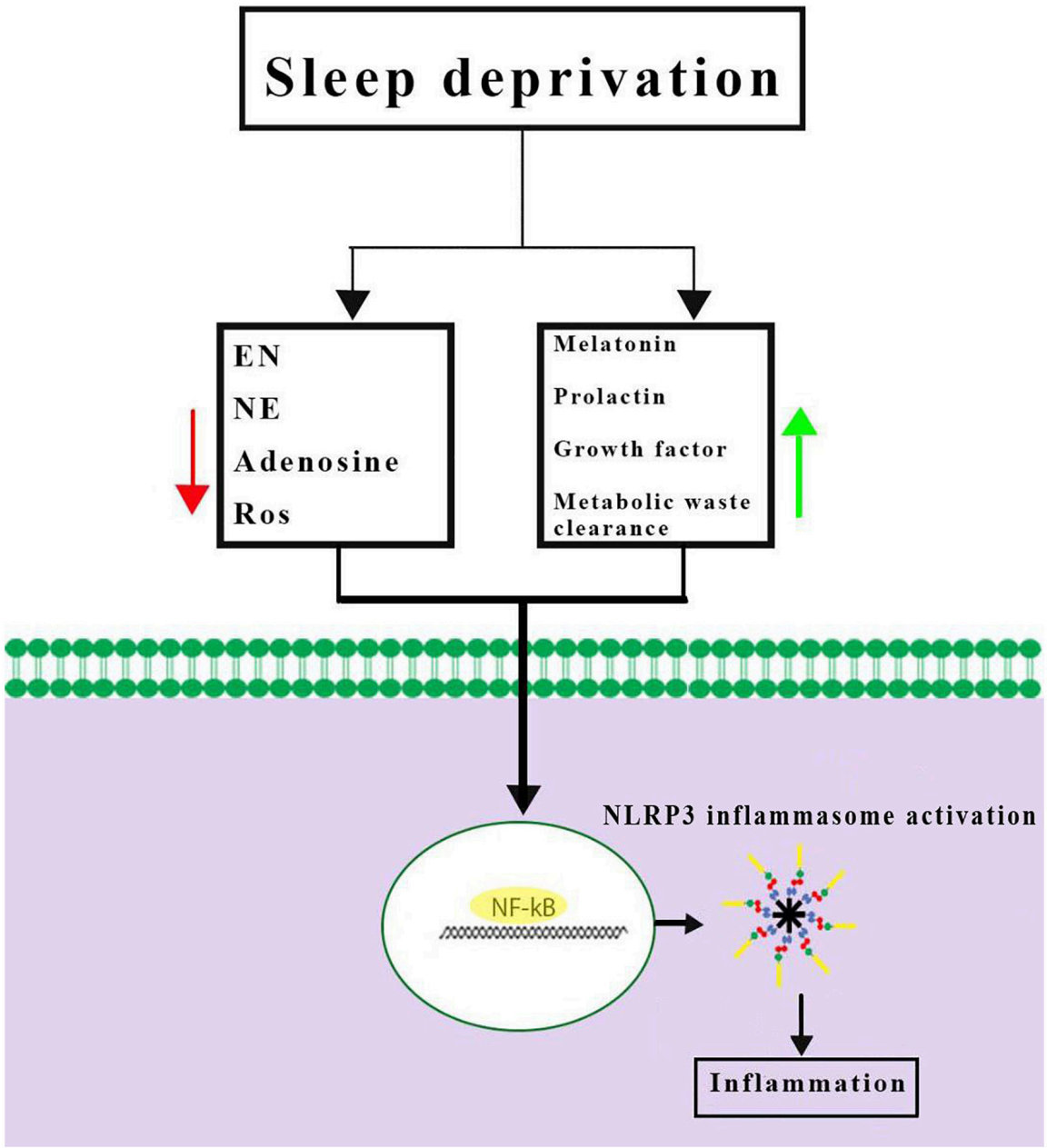


FIGURE 3
Possible mechanisms of Nod-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome activation caused by sleep deprivation. Sleep deprivation can change neuromodulators and neuro-endocrines involved in sleep regulation and they could be altered during and after sleep deprivation. Together, these results demonstrated a priming signal for NLRP3 inflammasome activation by activating the NF-κB signaling pathway. EN, epinephrine; NE, norepinephrine; ROS, reactive oxygen species.

However, the evidence is limited and conflicting, and more research is needed before any conclusions can be made. The P2 × 7 receptors are extracellular ATP-gated ion channels widely expressed in different cell types including blood, glial, neural, endothelial, muscle, and renal cells. These receptors also act as a second signal for NLRP3 inflammasome activation, resulting in the release of IL-1β and IL-18, and pyroptosis. Chronic SD may trigger depression by unbalancing neurochemicals such as serotonin in the brain. The likely cause for this is that ionotropic P2 × 7Rs increase the activation of NLRP3 inflammasome in the astrocytes, which result in development of depression-like behaviors caused by prolonged SD (6 h per day for 3–4 weeks). In addition, activated P2 × 7Rs reduce the expression of 5-HT2B receptors in astrocytes (Xia et al.,

2020). Antidepressant fluoxetine can increase signal transducer and activator of transcription 3 (STAT3) activity (caused by the reduced expression of 5-HT2B receptors) while suppressing the activation of NLRP3 inflammasome and avoiding SD-induced neurotoxicity (Xia et al., 2017). Moreover, it is yet unclear if fluoxetine can improve sleep quality and minimize the negative effects of SD.

## SD-induced anxiety: Effects of NLRP3 inflammasome inhibition

This idea that an immunological dysfunction plays a significant role in mental health dates back many decades. SD is linked to a variety of mood disorders, including depression and anxiety. It has been revealed that long lasting SD enhances the circulating concentrations of inflammatory factors, which are known as risk factors of anxiety development and progression (Manchanda et al., 2018). A new study has found that injection of flavanol-rich dietary preparation (FDP) to mice following persistent SD exposure results in anti-anxiety activity by pharmacological inhibition of microglia activation and NLRP3 inflammasome activity. Moreover, release of IL-1 is inhibited by FDP-derived metabolites, and NLRP3-deficient animals show anxiety reduction in response to prolonged sleep deprivation. These results indicate that FDP's anxiolytic effects are partly attributable to inhibition of NLRP3 activity. The same study also demonstrated that chronic sleep deprivation can alter the expression of circadian clock genes such as Bmal1 in the hippocampus, which plays a critical role in suppressing the expression of NLRP3 genes (Smith et al., 2021). These findings may explain how NLRP3 inflammasome is involved in neural networks and cognitive function pathways.

## Insomnia and NLRP3 inflammasome

Insomnia is described as having trouble falling asleep and staying asleep, or waking up too early in the morning. These sleep symptoms are accompanied with daily cognitive dysfunction including poor concentration, memory problems, learning disability, mood fluctuations, and so on. To be a person diagnosed with the insomnia disorder, a patient would have to report experiencing these symptoms at least three times a week and for a minimum period of 3 months. Insomnia is a relatively prevalent sleep disorder with a total population-based frequency of about 10% that is characterized with multiple etiologies as well as several subtypes (Rosenberg et al., 2021). Among different subtypes, the insomnia with objective short sleep duration (IOSSD) seems to be rapidly rising in recent years. The objective short sleep duration is commonly described as a continuous nocturnal sleep period of less than 6 h, as recorded by objective measurement techniques like polysomnography (PSG) (Fernandez-Mendoza et al., 2021). The latest research studies have demonstrated that chronic insomniacs are identified with overexpression of NLRP3, ASC, and caspase-1 in peripheral blood mononuclear cells (PBMCs) compared to controls. In addition, increased concentrations of NLRP3 plasma and activity of hypothalamic-pituitary-adrenal (HPA) axis are related to sympathetic hyperactivity. Epinephrine and norepinephrine are important catecholamines in the neuroendocrine system when sympathetic nervous system activity is dominant. A previous study demonstrated the activation of NLRP3 inflammasome in IOSSD patients and proposed that changes in epinephrine and norepinephrine levels in patients may be partly responsible for NLRP3 inflammasome activation *via* stimulation of leukocyte adrenergic receptors [e.g., Adrenoceptor Beta 2 (ADBR2) and Toll-like-receptor-4 (TLR-4)] and activation of NF-κB-mediated inflammatory signal pathway. In addition, studies have discovered that NLRP3 activity is positively related to short sleep duration and sleep fragmentation (Wang, 2020). In chronic insomnia patients, there is controversy in the findings that show the existence of a link between activation of NLRP3 inflammasome pathway and REM sleep duration (Wang et al., 2020; Aghelan et al., 2022).

## Sleep apnea syndrome and NLRP3 inflammasome

Sleep apnea syndrome is defined by episodes of breathing interruption during sleep, and the air-flow stoppage can last for over 10 s. There are three categories of sleep apnea called central, complex, and obstructive. Central sleep apnea happens when cessation in breathing occurs mainly due to lack of central nervous system's drive to the muscles to breathe during sleep. Complex sleep apnea is a combination of obstructive and central sleep apneas (Orr et al., 2017). Obstructive sleep apnea (OSA) is the most frequently occurring form, affecting approximately 14% of males and 5% of females and its highest prevalence is reported among middle-aged males. It occurs when complete or partial airway obstruction, caused by pharyngeal collapse during sleep, results in loud snoring or choking, intermittent hypoxemia, sleep fragmentation, and excessive daytime sleepiness (Osman et al., 2018). Recent studies have shown that OSA may increase the incidence of chronic kidney disease and acute kidney injuries. However, despite various undesirable side effects including dry mouth, increased number of awakenings, blocked-up nose, mask pressure, and mask leaks, continuous positive airway pressure (CPAP) is the most widely used therapy for this sleep disorder. Currently, no effective strategy is available for prevention of the renal injuries caused by OSA (Wang et al., 2020; Fernandez-Mendoza et al., 2021). One study indicated that

TABLE 1 Summary table of preclinical and clinical studies included in the present review study.

| # | Findings | References |
|---|---|---|
| **Preclinical studies** | | |
| 1 | The activation of the NLRP3 inflammasome can modulate sleep induced by wakefulness. These findings show that the NLRP3 inflammasome is an important mechanism involved in sleep responses to sleep loss and pathogen components in the brain. | (Zielinski et al., 2017) |
| 2 | The biological clock regulates NLRP3 expression and activation in diverse tissues. Circadian oscillations of NLRP3 signaling is lost in clock disruption models, which contributes to the development of disorders. | (Pourcet and Duez, 2020) |
| 3 | NLRP3 inflammasomes are involved in neurovascular coupling involving SWA. | (Zielinski et al., 2020) |
| 4 | The MAPK/NLRP3 axis may be important in the development of SD neuronal pyroptosis. The NLRP3 inflammasome is thought to be a potential therapeutic target for SD-induced neuroinflammation in the hippocampus. | (Fan et al., 2021) |
| 5 | SD modifies the expression of the circadian gene Bmal1, which controls NLRP3 expression and IL-1 production. FDP's anxiolytic effects may be mediated by inhibiting NLRP3 inflammasome activity. | (Smith et al., 2021) |
| 6 | The administration of the NLRP3 inhibitor (MCC950) prevents SD-induced changes in microglia morphology. | (Smith et al., 2019) |
| 7 | SD activates the NLRP3 inflammasome in neurons, astrocytes, and microglia, although this activation varies depending upon the brain area. | (Niznikiewicz et al., 2017) |
| 8 | Treatment with modafinil reduced inflammasome activity and neuronal pyroptosis *via* the NLRP3/NLRP1/NLRC4-caspase-1-IL-1β pathway. Targeting the regulation of impaired neuronal pyroptosis and neuroinflammation may be a promising therapeutic strategy for treatment of SD. | (Xiong et al., 2022) |
| 9 | The P2 × 7 receptors promote the formation of the NLRP3 inflammasome and the ATP-induced release of mature interleukin (IL)-1b and IL-18 from astrocytes, which leads to the development of chronic SD-induced depressive-like behavior. | (Xia et al., 2020) |
| 10 | Fluoxetine can prevent the activation of NLRP3 inflammasome and avoiding SD-induced neurotoxicity. Furthermore, the activation of STAT3 is an important target that regulated the expression of NLRP3 inflammasomes in an SD model. | (Xia et al., 2017) |
| 11 | This study identified the effects of rapamycin on OSA-associated renal injury. Inhibiting the mTOR signaling pathway by rapamycin can significantly reduce the levels of NLRP3 and organ damage caused by OSA. | (Liu et al., 2022) |
| 12 | MiR-224-5p reduces inflammation through the regulation of NLRP3 expression in T2DM With OSA, which finally regulated the NLRP3/IL1β pathway in the hippocampus. | (Du et al., 2020) |
| 13 | Mice lacking NLRP3 had attenuations in the significant increased amounts of NREM sleep and EEG delta power occurring 24 h after TBI and the significant reductions seen 2 months after TBI that were observed in wild-type mice. The findings suggest that NLRP3 inflammasomes contribute to dysregulated sleep occurring acutely or more persistently after TBI. | (Zielinski et al., 2021) |
| **Clinical studies** | | |
| 14 | Sleep fragmentation may contribute to dysregulation of NLRP3 inflammasome in IOSSD. Potential mechanisms linking sleep loss and NLRP3 inflammasome may include the activation of sympathetic system, hypothalamus-pituitary-adrenal axis and production of ROS. | (Wang et al., 2020) |
| 15 | NLRP3 inflammasome gene expression have a negative correlation with REM sleep duration. This evidence suggest that NLRP3 inflammasome is involved in the pathogenesis of the sleep disorders. inhibitors of the NLRP3 inflammasome may be promising therapeutic agents in sleep deprivation and sleep fragmentation. | (Aghelan et al., 2022) |
| 16 | There was no difference in the OSA development according to NLRP3 level. | (Kerget et al., 2021) |

pharmacological inhibition of mammalian target of rapamycin (mTOR)/NLRP3 axis by rapamycin, alleviates the OSA-induced renal damage (Liu et al., 2022). OSA-related type-2 diabetes mellitus (T2DM) is another frequent co-morbidity that affects nearly half of the adult patients and is associated with increased morbidity, mortality, and healthcare costs (Jehan et al., 2018). In recent years, microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) such as metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) have been proposed as new and crucial regulators of diverse biological processes (Vafadar et al., 2019). Mechanistically, it has been shown that NLRP3 inflammasome-mediated inflammatory responses are activated in T2DM animals with OSA as well as cell models, which may be related to MALT1 overexpression leading to inhibition of miR-224-5p in the hippocampus (Du et al., 2020). Most of the studies have focused on the intermittent hypoxemia and are limited by the absence of other cardinal features of OSA, including sleep fragmentation. In other studies investigating plasma NLRP3 levels in OSA patients, it has been observed that the levels of proinflammatory cytokines IL-1 and IL-18 are increased independent of NLRP3 levels, and this controversy remains to be elucidated (Kerget et al., 2021).

The take-home message of this research is that: (i) increased activity of the sympathetic nervous system and increased oxidative stress are considered as primary activators

of NLRP3 activation, which in turn, play an essential role in overproduction of potent proinflammatory cytokines, particularly IL-1 and IL-18. (ii) Knockout of NLRP3 genes or pharmacological blockage can alleviate OSA-associated neurocognitive impairment, pulmonary hypertension, cardiac injury, T2DM, and renal injury.

## Traumatic brain injury-induced sleep disturbance: Role of NLRP3 inflammasome

Sleep disturbance after traumatic brain injury (TBI), also known as intracranial injury, occurs when a sudden trauma damages the areas of the brain involved with controlling sleep patterns (Jehan et al., 2018; Liu et al., 2022). Sleep dysregulation is frequent after TBI, and approximately 30–70% of the patients experience sleep disturbances such as insomnia, daytime fatigue, and disruptions in their sleep-wake cycle (Vafadar et al., 2019; Du et al., 2020). Recent findings from human and rodent studies have indicated an upregulation in NLRP3-related molecules following TBI (Grima et al., 2016; Ismael et al., 2021). Other findings have suggested that NLRP3 inflammasomes contribute to deregulated sleep that occurs acutely or more persistently after TBI (Zielinski et al., 2021). A variety of rodent model studies have indicated that selectively suppressing the NLRP3 inflammasome can mitigate neuroinflammation and improve outcomes following TBI (Sandsmark et al., 2017; Mohammed et al., 2020). The studies included in the present review are summarized in **Table 1**.

## Conclusion and perspectives

In recent years, a growing body of evidence has emerged that demonstrates the importance of the mechanistic connections between NLRP3 and SD-induced detrimental consequences (**Supplementary Figure 1**), but there are still several pieces of the puzzle to be put in place. For ethical reasons, clinical studies cannot evaluate the expressions of NLRP3 inflammasome in the human brain, which can directly reflect the effects of sleep on neuroinflammation, and thus there is a need for more clinically relevant data. In animal research, issues and challenges can be seen from different viewpoints: (i) some animals show differential vulnerability to the effects of SD compared to humans. (ii) Additionally, the effects of sleep differ depending on the SD technique employed and the length of SD. Moreover, paying attention to the crosstalk of all the driving factors of NLRP3 inflammasome activation, such as inflammatory responses, autonomic control, oxidative stress, and endothelial function is highly recommended to prevent possible comorbidities, which are commonly seen in patients. In conclusion, NLRP3 inflammasome or its downstream pathways, is a potential target for therapy in order to improve the clinical outcomes of SD. However, the treatment process can be complicated due to the reciprocal and complex relationship of SD with NLRP3 inflammasome activation. Nonetheless, additional research is required to support such a causal claim and there are some factors to consider: First, why sleep deprivation occurs, and second, how do different types of SD (Total, REM, and NREM) contribute to the NLRP3 inflammasome activity.

## Author contributions

MA wrote the original text, revised, and assisted with editing the manuscript. ZY helped generate ideas for the framework of the manuscript, conducted the linguistic sorting investigation, and assisted with editing the manuscript. SG drew the figures and assisted with revising the manuscript. GH helped generate ideas for the framework of the manuscript and revised the manuscript. All authors contributed to and approved the final manuscript.

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2022.1018628/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Summary of the relationship between sleep deprivation (SD) and Nod-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome.

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# Associations between resting state brain activity and $A_1$ adenosine receptor availability in the healthy brain: Effects of acute sleep deprivation

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**Introduction:** Previous resting-state fMRI (Rs-fMRI) and positron emission tomography (PET) studies have shown that sleep deprivation (SD) affects both spontaneous brain activity and $A_1$ adenosine receptor ($A_1$AR) availability. Nevertheless, the hypothesis that the neuromodulatory adenosinergic system acts as regulator of the individual neuronal activity remains unexplored.

**Methods:** Therefore, fourteen young men underwent Rs-fMRI, $A_1$AR PET scans, and neuropsychological tests after 52 h of SD and after 14 h of recovery sleep.

**Results:** Our findings suggested higher oscillations or regional homogeneity in multiple temporal and visual cortices, whereas decreased oscillations in cerebellum after sleep loss. At the same time, we found that connectivity strengths increased in sensorimotor areas and decreased in subcortical areas and cerebellum.

**Discussion:** Moreover, negative correlations between $A_1$AR availability and rs-fMRI metrics of BOLD activity in the left superior/middle temporal gyrus and left postcentral gyrus of the human brain provide new insights into the molecular basis of neuronal responses induced by high homeostatic sleep pressure.

KEYWORDS

**$A_1$ adenosine receptor, acute sleep deprivation, resting-state fMRI, regional homogeneity, amplitude of low frequency fluctuations, degree centrality**

## 1. Introduction

Sleep deprivation (SD) is a tool to study negative consequences of high homeostatic sleep pressure on brain and behavior. By using functional magnetic resonance imaging (fMRI), several meta-analysis studies (Ma et al., 2015; Yeo et al., 2015; Javaheripour et al., 2019; Saletin et al., 2019; Ning et al., 2022) revealed SD-induced changes in neuronal activity of

brain regions, typically in the prefrontal cortex, thalamus, and intraparietal cortex regulating arousal, attention ability or emotional processing. Notably, several resting-state (Rs-)fMRI metrics, including amplitude of low-frequency fluctuations (ALFF) and its normalized version (fractional ALFF, fALFF), regional heterogeneity (ReHo), and degree centrality (DC), were applied to well characterize the differences in the regional properties of spontaneous brain activity (Zang et al., 2004, 2007; Zou et al., 2008; Zuo et al., 2012). In short, ALFF and fALFF are used to reflect the temporal fluctuations of low frequency oscillations (<0.1 Hz) across entire time series. ReHo is a voxel-based parameter for evaluating the similarity or synchronization of time series between a given voxel and its nearest neighbors. DC is an index of local functional connectivity (FC) strength and thus identifies the highest connected nodes by counting the number of direct connections to other nodes. Using these fMRI measures, we are capable to uncover voxel-based spectral and temporal characteristics of brain neuronal activity. For instance, prior Rs-fMRI studies (Gao et al., 2015; Wang et al., 2016; Chen L. et al., 2018) demonstrated reduced ALFF/fALFF values in the frontal and parietal cortex while increased values in the visual cortex and left sensorimotor cortex under the condition of sleep deprivation. Thus, the consistent changes across these fMRI properties should also be further investigated after acute sleep loss.

Generally, a neuronal response requires increased cellular energy metabolism that accelerates local cerebral blood flow or substrate delivery. With application of Positron Emission Tomography (PET), growing evidence (Wu et al., 1991; Thomas et al., 2000; Elmenhorst et al., 2007; Volkow et al., 2008, 2012; Qu et al., 2010; Holst et al., 2017) presented that acute SD remarkably upregulated $A_1$ adenosine receptor ($A_1$AR) availability, whereas glucose metabolism and dopamine D2/D3 neurons in human brain were reduced. Adenosine is a neuromodulator and directly linked to the energy metabolism as substrate of the breakdown of adenosine triphosphate. As a crucial mediator for promoting the homeostatic sleep drive, $A_1$AR can be found in widespread brain regions (Bjorness and Greene, 2009; Huang et al., 2011). Elmenhorst et al. (2017) used [$^{18}$F]-CPFPX PET and reported that sleep loss increased the $A_1$AR availability in some cortical and subcortical brain regions, including the temporal cortex, striatum and insula. Meanwhile, neuronal alterations of these brain regions were also investigated by previous fMRI studies (Bell-McGinty et al., 2004; Venkatraman et al., 2007; Gujar et al., 2011; Krause et al., 2019). For instance, one earlier study (Krause et al., 2019) confirmed that sleep loss extremely amplified pain reactivity within primary somatosensory cortex but reduced pain reactivity in higher-order evaluation and decision-making regions of the striatum and insula. For those above-mentioned overlapped regions, no neuroimaging studies investigated so far whether changes in their neuronal activity are associated with corresponding changes in $A_1$AR availability. Therefore, we aimed to evaluate the contribution of $A_1$AR in the specific regional properties of neural activities of some key brain regions by combing these two independent modalities.

Hence, our present study intends to explore the alterations in common Rs-fMRI metrics after 52 h of SD (SD52) and after 14 h of recovery sleep (RS14), as well as whether potential changes in these Rs-fMRI metrics are associated with changes in $A_1$AR availability and behavioral performance. To this end, we first conducted voxel-based statistical comparisons of ALFF/fALFF, ReHo, and DC after SD52 in comparison with RS14. Then, we correlated the differences (SD52 - RS14) in $A_1$AR availability with the differences in Rs-fMRI metrics of those significant clusters. Lastly, we tested the correlations between the differences (SD52 - RS14) in the above neuroimaging parameters and corresponding neuropsychological performance.

# 2. Materials and methods

## 2.1. Participants and study protocol

Fourteen young males (age: 28.21 ± 5.21 years, mean body mass index: 24.39 ± 3.58) were recruited. All participants were interviewed to ensure that they did not have any neurological or psychiatric diseases prior to the experiments. Each subject underwent PET, Rs-fMRI scans, and neuropsychological tests twice, at SD52 and at RS14 conditions (**Figure 1A**). Neuropsychological examinations included a 3-min version of psychomotor vigilance test (PVT), spatial 3-back working memory task, and sleepiness rating scale (Karolinska Sleepiness Scale, KSS). PVT-speed, PVT-lapses, 3-back hits, 3-back reaction time, as well as KSS sleepiness scores were derived. Details on inclusions of all participants, procedures of MRI/PET scans, and neuropsychological testing have been documented in the **Supplementary material** and our earlier publications (Elmenhorst et al., 2017; Li et al., 2020). This study was approved by the Ethics Committee of the Medical Faculty of the University of Düsseldorf and informed consent was obtained from all participants.

## 2.2. Rs-fMRI and [$^{18}$F]-CPFPX PET acquisitions

A 3T Siemens MAGNETOM Trio MRI scanner (Erlangen, Germany) with a 32-channel head coil was used to obtain MRI datasets. Subjects were instructed to keep the eyes open and to focus on a "+" that was presented on a screen positioned at the end of the gantry. By using a gradient-echo echo planar imaging sequence, we acquired Rs-fMRI datasets with following parameters: Time of Repetition (TR) = 2.2 s, Echo Time (TE) = 30 ms, Flip angle = 90°, matrix size = 64 × 64, 36 slices, slice thickness = 3.1 mm, voxel size = 3.1 × 3.1 mm$^2$, 146 volumes in total. Meanwhile, we conducted a 3D magnetization-prepared rapid acquisition gradient echo (MPRAGE) anatomical scans (TR = 2.25 s, TE = 3.03 ms, matrix size = 256 × 256, 176 slices, voxel size = 1 × 1 mm$^2$, slice thickness = 2.25 mm, flip angle = 90°).

For the collection of [$^{18}$F]-CPFPX PET datasets, we used a Siemens ECAT EXACT HR+ scanner (Siemens-CTI). The radiotracer was injected as a bolus followed by a constant infusion with a $K_{bol}$ value of 55 min. Scan duration was 100 min. Arterialized venous blood sampling was taken at minute timepoints 1, 5, and 10, and every 10 min subsequently.

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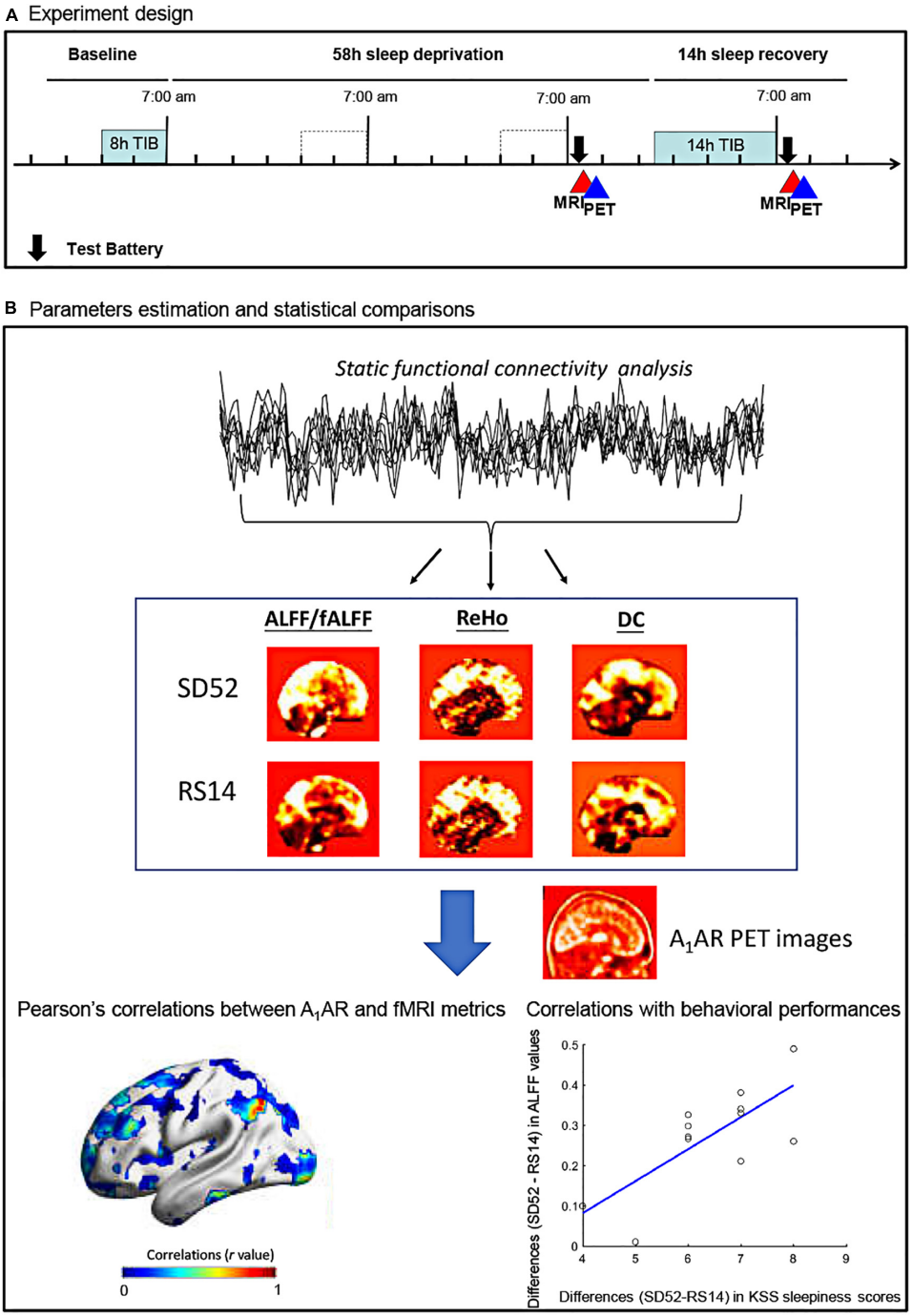


FIGURE 1
Flowchart of the study protocol for the 52 h of sleep deprivation. ALFF, amplitude of low frequency fluctuations; fALFF, fractional amplitude of low frequency fluctuations; ReHo, regional heterogeneity; DC, degree centrality; KSS, Karolinska Sleepiness Scale; SD52, 52 h of sleep deprivation; RS14, 14 h of recovery sleep; $A_1AR$, $A_1$ adenosine receptor; TIB, time in bed.

## 2.3. Rs-fMRI dataset preprocessing and analysis

Figure 1B showed the detailed flowchart of our study. All datasets were preprocessed using DPABI_V3.1[1] and SPM12 (7219).[2] In details, first 5 volumes were discarded to ensure that the subject has adapted to the scanner and to remove potential head movements. Secondly, we performed slice-timing and head motion correction. During this step, no subjects were excluded because inclusion criteria were fulfilled: head

1 http://rfmri.org/dpabi

2 http://www.fil.ion.ucl.ac.uk/spm/software/spm12/

transitions < 3 mm, rotations < 3° or mean Framewise Displacement (FD) value < 0.35 mm. As a result, mean FD values of two conditions were estimated as follows: SD52, 0.16 ± 0.06 mm; RS14, 0.15 ± 0.07 mm. Thirdly, Friston 24-motion parameters and signals from cerebrospinal fluid and white matter were regressed out. We then normalized the preprocessed Rs-fMRI images to the standard MNI template space using the DARTEL algorithm and removed a linear trend from the time series of each subject. Lastly, we performed band-pass filtering (0.01 ~ 0.08 Hz). No global signal regression was applied. To generate the parametric maps of ALFF/fALFF and DC, we smoothed the normalized Rs-fMRI images with 8 $mm^3$ Full Width at Half Maximum (FWHM). Given some specific frequency bands of spontaneous brain activity were thought to have distinct properties and physiological functions (Buzsaki and Draguhn, 2004; Zuo et al., 2010; Han et al., 2011), we additionally applied Slow-5 (0.01–0.027 Hz) and Slow-4 (0.027–0.073 Hz) to replicate our findings of ALFF/fALFF analysis.

In details, the power spectrum in three frequency ranges of 0.01–0.08 Hz, Slow-5 and Slow-4 range were separately computed at whole-brain voxel-wise level. We then *z*-transformed the ALFF values before the statistical analyses. The fALFF were conducted by their rations of ALFF values relative to full frequency range (0–0.25 Hz).

For other parameters, we exhibited the equations:

$$DC\,(i) \;=\; \int_{j\,=\,1}^{N} \mathrm{a}_{ij} \tag{1}$$

In a weighted graph, the element $a_{ij}$ indicates the connection or edge from a specific node *i* to its connecting node *j*.

$$W \;=\; \frac{\sum (R_i)^2 - \mathrm{n}(\overline{R})^2}{\frac{1}{12}K^2(n^3 - n)} \tag{2}$$

where *W* is the Kendall's coefficient of concordance among given voxels, ranged from 0 to 1; *R*i is the sum rank of the $i^{th}$ time point; where $\overline{R}$ is the mean of the (n+1)K/2; *K* is the number of time series within a measured cluster ($K = 27$ in our study).

## 2.4. PET preprocessing and calculations

We performed realignment, coregistration, segmentation, and normalization of the PET dataset to standard MNI152 space with PMOD (v3.305, PMOD Group). The total distribution volume $V_T$ in the equilibrium (between 50 and 100 min) is calculated as $V_T = \mathrm{TAC}/C_p$, with TAC represents the tissue activity

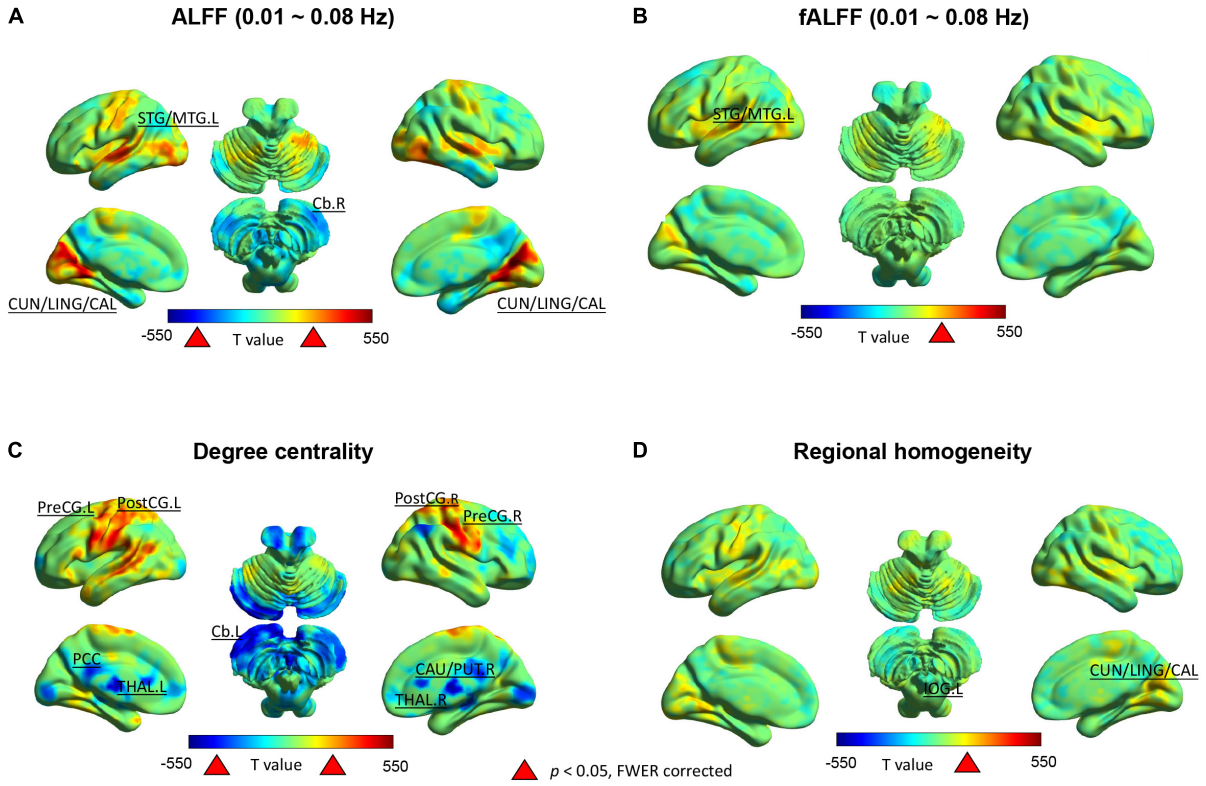


FIGURE 2
Between-group differences (52 h of sleep deprivation–14 h of recovery sleep) in the Rs-fMRI metrics for 14 healthy participants. Positive *t*-value indicates the significant increases and negative *t*-value represent decreases after 52 h of sleep deprivation. ALFF, amplitude of low frequency fluctuations; fALFF, fractional amplitude of low frequency fluctuations; L, left hemisphere; R, right hemisphere; STG/MTG, superior/middle temporal gyrus; CUN/LING/CAL, cuneus/lingual/calcarine; Cb, cerebellum; PostCG, postcentral gyrus; PreCG, precentral gyrus; PCC, posterior cingulate cortex; CAU/PUT, caudate/putamen; THAL, thalamus.

concentration of the radioligand and $C_p$ indicates plasma activity of parent compound (Elmenhorst et al., 2007). After identifying the significant clusters of local Rs-fMRI metrics, we extracted their corresponding mean $A_1$AR distribution volumes for each region. During the voxelwise correlations analysis, we smoothed the PET dataset with 8 $mm^3$ FWHM to increase the spatial coherence.

## 2.5. Statistical comparisons

Considering the small sample size, we applied a Permutation Analysis of Linear Models approach (PALM, 5000 permutations) (Winkler et al., 2014) to perform two-sample paired *t*-test across all local Rs-fMRI metrics. It should be noted that a permutation test with Threshold-Free Cluster Enhancement (TFCE) approach reached the best balance between false wise error rate (FWER) and test–retest reliability in an earlier study (Chen X. et al., 2018). Hence, the significant threshold was determined at one-tailed FWER $< 0.05$ with minimum cluster size $> 10$ voxels, which ran in DPABI_V3.1 toolbox. During the statistical comparisons, mean FD values were carried out as variance of no interest.

## 2.6. Voxelwise and regional-based correlations analysis

Owing to the failures of acquiring one of the two PET images in two participants, we only retained 12 participants for further correlation analysis. We extracted the mean $A_1$AR values for those significant clusters in the fMRI properties and calculated the differences (SD52 - RS14) for each subject. Using a combination of PALM (5000 permutations) and TFCE approaches, we computed the whole brain voxelwise Pearson correlation between the differences (SD52 - RS14) in $A_1$AR distribution volumes and the differences in each of Rs-fMRI metrics. Statistical threshold was determined at one-tailed FWER $< 0.05$. For the region of interest (ROI)-based analysis, we used Pearson correlation to investigate the associations between differences (SD52 - RS14) in $A_1$AR distribution volumes of those significant clusters and the differences in corresponding Rs-fMRI metrics. Finally, we applied Pearson correlations to separately examine the relationships of the differences (SD52 - RS14) in local Rs-fMRI metrics and $A_1$AR distribution volumes with the differences in neuropsychological tests (KSS sleepiness scores, PVT-lapses, PVT-speed, and 3-back hits).

# 3. Results

## 3.1. Sleep deprivation-induced alterations in local Rs-fMRI metrics

In the typical frequency band (0.01 ∼ 0.08 Hz), we observed significantly higher ALFF values in the bilateral cuneus/lingual/calcarine and left superior/middle temporal gyrus, but lower values in the right cerebellum after SD52 compared to RS14 (**Figure 2A**). We only observed significantly increased fALFF values in the left superior/middle temporal gyrus (**Figure 2B**). As illustrated in **Figure 2C**, acute sleep loss significantly increased DC values in the bilateral precentral gyrus and postcentral gyrus, whereas DC values were significantly decreased in the thalamus, posterior cingulate cortex, right putamen/caudate, and left cerebellum. Regarding the ReHo maps, the values of the bilateral cuneus/lingual/calcarine were significantly higher after SD52 compared to RS14 (**Figure 2D**).

In the Slow-5 frequency band, only the ALFF values in the right cerebellum were significantly lower (**Supplementary Figure 1A**). However, in the Slow-4 band significantly increased ALFF was found in the bilateral superior/middle temporal gyrus, bilateral cuneus/lingual/calcarine, left inferior occipital gyrus, and right fusiform gyrus (**Supplementary Figure 1B**). For the fALFF maps in the typical frequency band and Slow-4, we found significantly higher values in the left superior/middle temporal gyrus after SD52 (**Supplementray Figure 1C**).

## 3.2. Correlation analysis between $A_1$AR availability and Rs-fMRI metrics

For the ROI-based comparisons, the differences in $A_1$AR distribution volumes within the left superior/middle temporal gyrus correlated negatively with the differences in mean ALFF values of the typical frequency band and Slow-4, as well as mean fALFF values of Slow-4 ($r = -0.685$, $p = 0.014$; $r = -0.69$, $p = 0.013$; $r = -0.82$, $p = 0.001$; **Figure 3**). The differences in $A_1$AR distribution volumes of left postcentral gyrus correlated negatively with the differences in DC values ($r = -0.67$, $p = 0.017$; **Figure 3**).

For voxelwise comparisons with FWER correction, we did not find any significant correlations between the differences (SD52 - RS14) in $A_1$AR distribution volumes and the differences in Rs-fMRI metrics (**Supplementary Figure 2**).

## 3.3. Correlations with neuropsychological tests

We detected that the differences (SD52 - RS14) in mean ALFF values (0.01 ∼ 0.08 Hz) and DC values of the PCC area were negatively correlated with the differences of PVT-speed (**Supplementary Table 1**, $r = -0.67$, $p = 0.009$; $r = -0.75$, $p = 0.002$). The differences (SD52 - RS14) in mean ALFF values of the left superior/middle temporal gyrus, mean fALFF values of the left middle temporal gyrus, and DC values of the left postcentral gyrus correlated positively with the differences in KSS sleepiness scores ($r = 0.63$, $p = 0.016$; $r = 0.54$, $p = 0.047$; $r = 0.71$, $p = 0.004$). However, the differences in DC values of the posterior cingulate cortex and left cerebellum correlated negatively with the differences in KSS sleepiness scores ($r = 0.71$, $p = 0.004$; $r = -0.74$, $p = 0.002$). The differences of mean ALFF values of the typical frequency and Slow-4 of the cuneus/lingual/calcarine area were negatively correlated with the differences of 3-back rection times ($r = -0.54$, $p = 0.045$; $r = -0.54$, $p = 0.047$). Differences in $A_1$AR availability in several of these identified brain regions correlated with the differences in PVT-lapses, 3-back hits, and

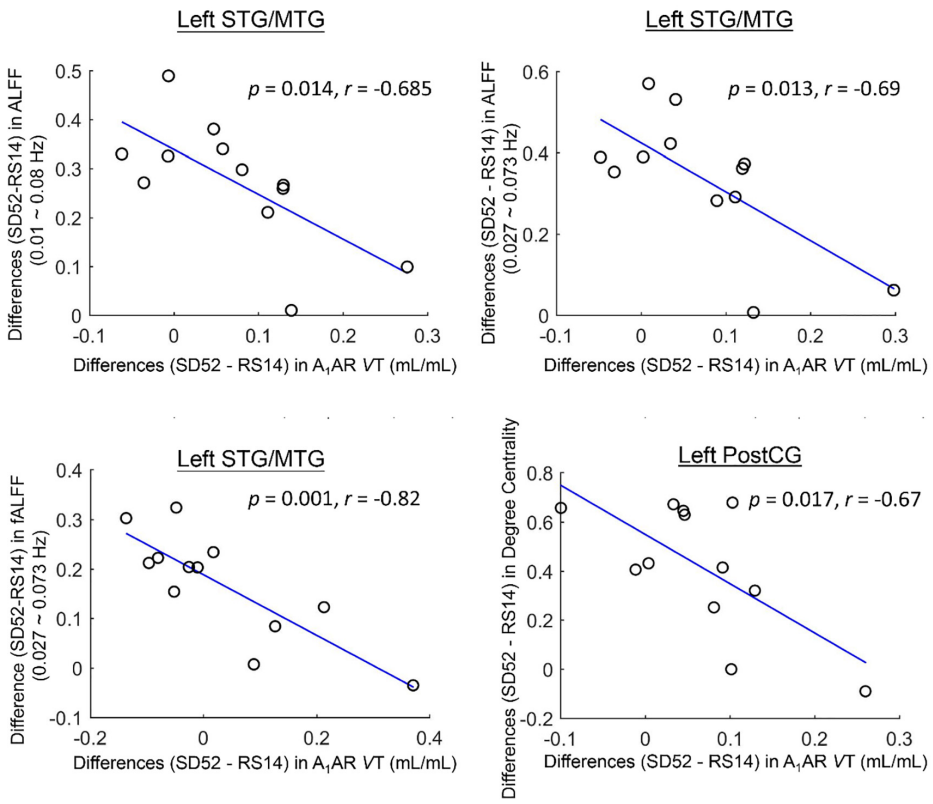


FIGURE 3
Cluster-based associations between the differences (52 h of sleep deprivation−14 h of recovery sleep) in $A_1$AR distribution volumes and Rs-fMRI metrics for 12 healthy participants. STG/MTG, superior/middle temporal gyrus; PostCG, postcentral gyrus; ALFF, amplitude of low frequency fluctuations; fALFF, fractional amplitude of low frequency fluctuations; VT, distribution volume.

KSS sleepiness scores (please refer to **Supplementary Table 2** for more details).

## 4. Discussion

This study systematically performed whole brain voxel-wise comparisons across local Rs-fMRI metrics of spontaneous brain activity (ALFF/fALFF, ReHo, and DC) comparing SD52 to RS14. After acute sleep loss, we identified both significant higher amplitude of low-frequency fluctuations (ALFF) and ReHo values in cuneus/lingual/calcarine. Meanwhile, ALFF values increased significantly in the superior/middle temporal gyrus, whereas they decreased in right cerebellum. Our findings also showed significantly higher degree centrality in bilateral precentral gyrus and postcentral gyrus, but decrease in thalamus, posterior cingulate cortex, right caudate/putamen, and left cerebellum. Furthermore, our correlation analysis suggested an increase in the differences (SD52 - RS14) of $A_1$AR availability were correlated with a decrease in the differences of mean ALFF/fALFF in left/middle temporal gyrus and degree centrality in left postcentral gyrus. An increase in the differences (SD52 – RS14) of local Rs-fMRI metrics within several brain regions significantly correlated with an impairment in PVT-speed and high feelings of subjective sleepiness, whereas lower $A_1$AR availability of most brain regions were associated with larger impairments in cognitive performances (PVT-lapses and 3-back hits).

Of these findings, the most prominent one is that high homeostatic sleep pressure synchronized amplitude of low frequency fluctuations of blood-oxygen-level-dependent (BOLD) activity in primary visual cortex, which was reflected by the increased ALFF/fALFF and ReHo in bilateral cuneus/lingual/calcarine after sleep loss. Similar results of higher ALFF values after 24 or 72 h of sleep loss have already been reported in two Rs-fMRI studies (Gao et al., 2015; Wang et al., 2016). Meanwhile, we also noticed the remarkably decreased degree centrality in this region, which is consistent with earlier findings (Farahani et al., 2019; Xu et al., 2021). After reviewing the behavioral consequences in 21 studies, one earlier study (Waters et al., 2018) concluded that sleep restriction mainly deteriorated the visual domains (90% of these studies) in healthy participants, such as metamorphopsia, illusions, and hallucinations. Additionally, one fluorodeoxyglucose (FDG)-PET study (Thomas et al., 2000) identified a decreased cerebral metabolic rate of glucose uptake within this brain area. In the frequency band of Slow-4, our results extended the sleep-loss related changes to the right fusiform gyrus and left inferior occipital gyrus, which were discovered in other fMRI studies as well (Gao et al., 2015; Chen L. et al., 2018). Though the nature and their pathological functions of low frequency bands were not fully identified yet, some brain regions are more sensitive to one of these two different neighboring frequency bands in the

neuroimaging studies of human brain (Hutchison et al., 2004; Han et al., 2011; Li et al., 2017). Combined with their correlations with outcomes of PVT, we propose that the upregulated power spectrum of BOLD signals in these visual areas primarily delayed the response speed in the attentional task. Meanwhile, our results revealed increased ALFF/fALFF values in bilateral superior/middle temporal gyrus but attenuated ALFF values in right cerebellum after sleep loss. Using single-neuron recordings in the human neurosurgical patients, one study (Nir et al., 2017) that reported that sleep deprivation induced prolonged and weakened spiking responses of individual neurons in middle temporal gyrus prior to cognitive lapses during a face/non-face categorization PVT. Lastly, the reduced power spectrum of BOLD activity within the right cerebellum, which was in line with a prior study (Chen L. et al., 2018), may reflect the accelerated oxygen consumption in order to sustain movement, emotional and cognitive functions.

Using the index of degree centrality, we identified hyperconnectivity within the sensorimotor cortex (PreCG and PostCG), but reduced strengths in some hub regions of the default-mode network (PCC) and subcortical network (thalamus and CAU/PUT), as well as left cerebellum. After sleep deprivation, earlier study (Gorgoni et al., 2014) observed enhanced excitability with an amplitude increase of somatosensory evoked potentials. Moreover, they found that voltage changes correlated with post-SD fluctuations of subjects' sleepiness. Meanwhile, the decrease of thalamocortical connectivity after sleep loss has been shown to be critical for attention and arousal regulation in previous studies (Portas et al., 1998; Chee et al., 2008; Tomasi et al., 2009; Shao et al., 2013; Liu et al., 2018). More specifically, thalamic activity was decreasing with lower arousal level during resting-state but conversely elevated when the subjects were required to perform an attention task. Notably, reductions of FC strengths between thalamus and cortical areas were reported under the conditions of coma, general anesthesia, and non-rapid eye movement sleep (Kaufmann et al., 2006; Akeju et al., 2014; Picchioni et al., 2014; Hannawi et al., 2015). Therefore, these findings suggest that the thalamus acts as "control switch" to regulate human brain consciousness states. In the end, the FC strengths within the posterior cingulate cortex, right PUT/CAU of basal ganglia and left cerebellum were declined because of post-SD sleepiness, which was consistent with previous findings (Lazarus et al., 2013; Tomasi et al., 2016). To sum up, higher connectivity strengths within sensorimotor cortex but a decrease in strength in the default mode and subcortical network after sleep loss were closely associated with attentional deficiencies and sleepiness.

Most importantly, our findings highlight the negative associations between $A_1$AR distribution volumes and Rs-fMRI metrics in mean ALFF/fALFF values of left superior/middle temporal gyrus and degree centrality of left postcentral gyrus. To our knowledge, this is the first study to investigate their associations by combining PET and Rs-fMRI datasets whereas prior work was limited to separate investigations of the brain's $A_1$AR availability and BOLD activity (Gao et al., 2015; Elmenhorst et al., 2017). Noticeably, the $A_1$AR availability of superior/middle temporal gyrus were elevated in terms of some antidepressant therapies in the patients with major depression disorders, such as electroconvulsive therapy, deep brain stimulation, or transcranial magnetic stimulation (Sakagami et al., 2005; Bekar et al., 2008; Kato, 2009; Hamani et al., 2010). These findings may further indicate that the effectiveness of sleep restriction on emotional regulation of depressive patients might be accomplished by the accumulation of $A_1$ adenosine receptors density, which was correlated to slower oscillations of BOLD activity. With respect to the left postcentral gyrus, the decreases in $A_1$AR availability are associated with reduced FC strengths between sensorimotor cortex and other brain regions. Additionally, significant associations of $A_1$AR availability with PVT-lapses and 3-back hits indicate the high $A_1$AR bindings within these brain areas might be the molecular mechanism of spatial neglects during sustained attention, which was discussed in a previous publication (Elmenhorst et al., 2017).

Nevertheless, the sample size ($N$ = 14) of our study is relatively small and hence restricted the statistical power for detecting between-conditions differences. To solve this issue, we performed Permutation Analysis Linear Model with 5000 repetitions to produce the statistical inferences. Secondly, we should keep in mind that the neuronal or metabolic activity of the human brain after recovery sleep may not be fully restored to rested baseline level (Wu et al., 2006; Chai et al., 2020). Lastly, several PET studies (Wu et al., 1991; Chuah and Chee, 2008; Volkow et al., 2008, 2012) reported that acute sleep deprivation reduced glucose uptake, D2/D3 neurons, but increased cholinergic neurons in some brain areas. Therefore, the complex relationships among the BOLD activity, different types of neurotransmitters and behavioral outcomes should be investigated further.

## 5. Conclusion

In our current study, for the first time, negative correlations between $A_1$AR availability and BOLD activity in the left superior/middle temporal gyrus and left postcentral gyrus of the human brain provide new insights into the molecular basis of neuronal responses induced by high homeostatic sleep pressure.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of the Medical Faculty of the University of Düsseldorf. The patients/participants provided their written informed consent to participate in this study.

## Author contributions

CL conceived the presented idea, performed the computations, and wrote the manuscript. DE and E-ME designed and carried out the experiments and revised the manuscript. TK, AM, DA, and AB discussed the results and contributed to the final version of manuscript. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

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# Temporary amnesia from sleep loss: A framework for understanding consequences of sleep deprivation

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Throughout its modern history, sleep research has been concerned with both the benefits of sleep and the deleterious impact of sleep disruption for cognition, behavior, and performance. When more specifically examining the impact of sleep on memory and learning, however, research has overwhelmingly focused on how sleep following learning facilitates memory, with less attention paid to how lack of sleep prior to learning can disrupt subsequent memory. Although this imbalance in research emphasis is being more frequently addressed by current investigators, there is a need for a more organized approach to examining the effect of sleep deprivation before learning. The present review briefly describes the generally accepted approach to analyzing effects of sleep deprivation on subsequent memory and learning by means of its effects on encoding. Then, we suggest an alternative framework with which to understand sleep loss and memory in terms of temporary amnesia from sleep loss (TASL). The review covers the well-characterized properties of amnesia arising from medial temporal lobe lesions and shows how the pattern of preserved and impaired aspects of memory in amnesia may also be appearing during sleep loss. The view of the TASL framework is that amnesia and the amnesia-like deficits observed during sleep deprivation not only affect memory processes but will also be apparent in cognitive processes that rely on those memory processes, such as decision-making. Adoption of the TASL framework encourages movement away from traditional explanations based on narrowly defined domains of memory functioning, such as encoding, and taking instead a more expansive view of how brain structures that support memory, such as the hippocampus, interact with higher structures, such as the prefrontal cortex, to produce complex cognition and behavioral performance, and how this interaction may be compromised by sleep disruption.

KEYWORDS

**sleep deprivation, amnesia, memory, binding, decision-making**

## 1. Introduction

There is a considerable history of interest in the relationship between sleep and memory, but since the seminal studies on sleep and memory (Jenkins and Dallenbach, 1924), most research addressing this relationship has focused on how sleep strengthens memories established during previous waking hours. More recently, there has been increased study of the ability to learn and remember new information while sleep deprived. There are good reasons, both practical and

theoretical, to better understand how sleep deprivation may impair or degrade the quality of memory. With epidemic levels of insufficient sleep in modern industrial societies (Chattu et al., 2018), the potential for impaired learning due to lack of sleep is very high in schools and training environments. From a fundamental research perspective, if we are to identify the mechanisms by which sleep deprivation affects cognitive performance in general, memory is a critical domain to understand because what we remember from our experiences affects how we relate to others and how we make choices. Recognizing the much larger body of sleep research on how sleep strengthens previously acquired memories, it is noteworthy that Newbury et al. (2021) found that sleep deprivation after learning produced substantially smaller effects than a night of sleep deprivation before learning new material.

The effects of sleep deprivation before or after learning are typically mapped onto a division of memory processing into three phases: encoding, consolidation, and retrieval. Research on sleep deprivation after learning is most concerned with the role of sleep in memory consolidation. According to the *active systems view* of the consolidation of memory, sleep facilitates the transfer of information from hippocampally dependent processing more broadly to the neocortex where it is integrated with prior knowledge (Takashima et al., 2009; Walker, 2009; Born and Wilhelm, 2012; Rasch and Born, 2013). Research on sleep deprivation before learning analyzes possible impairments in the other phases of memory. The consensus from this research is that sleep deprivation degrades the capacity for encoding new information, and similar to studies of memory consolidation, has focused on the hippocampus and associated structures where sleep loss decreases hippocampal activation and alters its connectivity with other brain regions. Specifically, neuroimaging studies indicate that sleep deprivation decreases connectivity with the prefrontal cortex (PFC), temporal, and parietal lobes, and increases connectivity with alertness networks that include the thalamus (Yeo et al., 2015; Zhao et al., 2019; Chai et al., 2020; Gisabella et al., 2020).

Our purpose here is to provide a framework for better understanding the growing body of evidence on the effects of sleep deprivation before learning. Although there are typically large effects of sleep deprivation on subsequent learning, multiple investigators have noted that there is substantial variation in these effects for reasons that are as yet unknown (Cousins and Fernández, 2019; Vaseghi et al., 2021). We believe that lessons from the history of studies of memory impairment from hippocampal lesions, which produce anterograde amnesia, suggest that viewing sleep deprivation as producing a deficit in "encoding capacity" may be misleading and may obscure important connections between memorial effects of sleep loss and other cognitive processes not typically considered in a memory context.

## 2. Lessons from the study of amnesia

One of the most famous patients in the history of clinical neurology, Henry Molaison, known in the scientific and medical literature by his initials H.M. until after his death, provided the most important case study of profound anterograde amnesia resulting from bilateral resection of the medial temporal lobes (MTL). Study of H.M. had an extraordinary influence on subsequent memory research. This influence, and that of converging studies of other individuals with hippocampal damage, has been widely discussed and we need not provide a comprehensive review here (*cf.*, Scoville and Milner, 1957; Salat et al., 2006; Squire, 2009; Squire and Wixted, 2011). What is important for present purposes is to briefly review changes over time in conceptions of anterograde amnesia, and the role of the hippocampus in memory, as a basis for comparison to current research on sleep deprivation.

Studies of individuals with MTL lesions showed a striking dissociation between an apparent inability to learn new information and intact abilities in short-term memory (STM) and general intellectual functioning. In addition, deficits in memory produced by damage to the hippocampus were not accompanied by deficits in acquiring skills such as mirror drawing and tactile maze learning, or for showing automatic priming of perceptual or conceptual relations among words (Milner et al., 1968; Cohen and Squire, 1980; Levy et al., 2004). Thus, it was concluded that the hippocampus and related structures in the MTL are critical for the transfer of consciously accessible information in short-term memory to more permanent storage in long-term memory (Wickelgren, 1968; Baddeley and Warrington, 1970; Milner et al., 1998; Ranganath and Blumenfeld, 2005). This classic conception of the nature of anterograde amnesia is consistent with the viewpoint that impaired functioning of the hippocampus during sleep deprivation diminishes the capacity to encode new, explicit information. However, as research on amnesia has grown, and with the introduction of functional imaging of the hippocampus during memory tasks, new ideas have supplanted the original conception that the hippocampus functions to transfer information from STM to long-term memory (LTM).

Evidence accumulated from the study of LTM deficits in amnesia clearly demonstrates that the hippocampus supports *relational binding*, i.e., the linking of stimulus elements into integrated representations (Cohen and Eichenbaum, 1993; Ryan et al., 2000), with converging support obtained from animal studies and neuroimaging (Olsen et al., 2012; Bird, 2017; Schwarb et al., 2019). Hippocampally dependent binding occurs across various levels of cognitive processing, from perception to memory (Treisman and Gelade, 1980; Cohen and Eichenbaum, 1993; Yonelinas, 2013). Within the memory domain, binding primarily links stimuli with each other (e.g., associating pairs of words together; Yonelinas et al., 2001), or links stimuli with their relevant contextual information (e.g., remembering when or where an image was viewed; Mitchell and Johnson, 2009). Regardless of what is being bound, successful binding depends on having intact hippocampal functioning, particularly for the creation of complex, high-resolution bindings that help form more precise, differentiated memories (Yonelinas, 2013; Ekstrom and Yonelinas, 2020).

The evidence for relational memory problems in anterograde amnesia comes from a variety of paradigms and stimulus types. For example, MTL damage produces profound deficits in acquiring arbitrary associations of names and pictures (Morrow et al., 2020), binding of objects to the scenes they appear in (Hannula et al., 2015), and the binding of objects to their spatial locations (Horecka et al., 2018). However, it is important to note that the binding problems created by MTL damage will not only be manifest in tests of memory specifically targeting the relationships among stimulus items. Consider the well-known dissociation in which amnesia patients show deficits on explicit memory even as they show unimpaired performance on several tests of implicit memory (Graf et al., 1985). Explicit memories

are those that can be consciously and deliberately remembered, such as memories for events and experiences. In contrast, implicit memories are those that are manifest in performance (Squire and Dede, 2015) without awareness, such as habit-based skills or priming, i.e., the influence of stimuli that occurs unconsciously (Schacter, 1987; Squire, 2004; Squire and Dede, 2015). In general, explicit tests of memory will necessarily be dependent on binding of items with their context. Even if asked to simply recognize whether a word was in a study list or not, the context of studying the item in a particular list at a particular time is part of the information that supports a judgment based on recollection of studying the item and, at least for many types of stimuli, whether the word seems familiar (Squire and Wixted, 2011; Bird, 2017). Moreover, the role of the hippocampus in binding also means that its function cuts across the traditional three phases of encoding, consolidation, and retrieval. The associations bound together during encoding are critical to later retrieval because they allow for reinstating earlier hippocampal and cortical activation, even if only partial cues to the original experience are available (Henke, 2010). Reinstating hippocampal-cortical interactions likewise appears to play a role in consolidation (Murty et al., 2017; Cowan et al., 2021).

Although implicit memory is *generally* preserved in amnesia, problems with binding of stimulus elements have also been demonstrated under conditions of implicit memory rather than conscious recollection. Ryan et al. (2000) presented a series of scenes to control subjects and people with amnesia and monitored eye movements as the scenes were viewed. Some scenes were repeated in their original form and other scenes were repeated but with manipulation of the relationships among objects (e.g., shifting the position of one of the objects). Among control subjects, simple repetition reduced visual sampling of the objects in the scene, but manipulated scenes increased viewing time of the changes that were made. These eye movement effects occurred in the absence of conscious awareness of the scene changes. Subjects with amnesia showed the same effect as controls for simple scene repetition, but unlike controls, they showed no effect of changed scenes on viewing times. Thus, people with amnesia, who show severe deficits in conscious recollection, also, in an implicit memory procedure, failed to bind separate objects together in their memory representations.

Additional support for the role of the hippocampus in binding can also be found in studies of amnesia and motor memory. Early studies of the learning of motor procedures through practice, such as mirror drawing and pursuit rotor performance, suggested that motor memory was intact in patients with amnesia (Milner, 1962; Brooks and Baddeley, 1976). While motor skill learning can often occur without hippocampal involvement, particularly in the case of more implicit tasks, subsequent research showed that the hippocampus is critical to such memory when it involves learning higher order motor sequences (Albouy et al., 2013). For example, in the serial reaction time task (SRTT), people use visual cues to anticipate and reproduce a set of corresponding sequential motor responses (Chafee and Ashe, 2007). When the series can be learned from simple pairwise associations among the pattern of cues, amnesic patients do not show learning deficits, but when the pattern depends on more complex higher-order associations among the cues, the hippocampus is needed to bind the motor responses into a sequence. Therefore, if higher order relations must be bound, a process that is hippocampally dependent, amnesic patients show deficits in performance (Curran, 1997; Robertson, 2007).

The classic view that anterograde amnesia represents a failure of encoding information into LTM has been further undermined by studies showing that hippocampal damage disrupts some aspects of STM processing, and not just storage in LTM. For example, when amnesic patients are presented with scenes and are given a recognition memory test after only a few seconds, their memory for which objects are in the scene is typically intact, but memory for item locations within the scenes is very impaired (Olson et al., 2006; Yee et al., 2014). This pattern has been replicated with multiple types of associations among distinct elements including faces and scenes, colors and locations, and colors and numbers (see Olsen et al., 2012 for a review). These results suggest that a critical role of the hippocampus is to bind distinct elements in the focus of attention together into a composite representation that captures the temporal, spatial, and conceptual relationships among the elements (Cohen and Eichenbaum, 1993; Rubin et al., 2017). Results from studies of amnesic patients have received converging support from neuroimaging studies of hippocampal engagement during perceptual processing and working memory tasks (Ranganath and D'Esposito, 2001; Ranganath et al., 2005; Riggs et al., 2009).

Perhaps the most striking departure from the classic view of the hippocampus and amnesia comes from recent research demonstrating that the hippocampus is not limited to functions traditionally designated as being in the memory domain (Olsen et al., 2012; Rubin et al., 2017). Through its role in binding stimulus items together with their context, and through its interaction with other brain areas, particularly the PFC, the hippocampus makes an essential contribution to decision-making, spatial navigation, and some aspects of language use. Amnesia patients once thought to have a circumscribed memory deficit actually show other kinds of deficits related to binding and comparing information (Biderman et al., 2020). For example, performance on the Iowa Gambling Task (IGT), which was originally developed to understand decision-making deficits associated with damage to the ventromedial PFC (Bechara et al., 1994), is impaired in people with MTL damage as well (Bechara et al., 1994; Gutbrod et al., 2006). In the IGT, subjects choose cards from among four decks, and based on the outcomes associated with choices of each deck, they must learn which deck choices are advantageous and which are disadvantageous in the long run. There are multiple possible reasons for poor performance on the IGT, including an insensitivity to future consequences of choices (Bechara et al., 1996). However, in the case of amnesia patients, failure to develop an advantageous choice strategy is likely because the task involves associating gains and losses with their respective decks, i.e., a fundamental binding problem (*cf.*, Whitney and Hinson, 2012).

Further evidence that amnesia patients have difficulties in decision-making comes from Bakkour et al. (2019) who reported deficits in value-based decision-making among these patients. Although the patient group performed similarly to controls on a color discrimination task using familiar food items, when asked to compare food items and choose which one they would prefer, a difference between groups emerged. The patient group made choices that were less consistent with their initial evaluation of individual items, while control subjects made value-based comparisons that were highly consistent with initial evaluation of each of the individual items.

In summary, research on amnesia provides compelling evidence that the hippocampus, the functioning of which is known to be strongly affected by sleep deprivation, plays a critical role in LTM

through relational binding of stimuli and their context, while also functioning to bind items together in novel associations on timescales operating in perception and working memory. Further, hippocampal binding is needed for performance on several aspects of complex cognition typically considered outside the domain of memory research. We next evaluate whether deficits in these processes associated with anterograde amnesia may underlie sleep deprivation effects on memory and other cognitive processes.

## 3. Temporary amnesia from sleep loss

Based on strong evidence that the hippocampus plays a critical role in relational binding, along with the demonstrated effects of sleep deprivation on hippocampal functioning, we believe it is useful to think of sleep loss effects on memory, and some other aspects of cognition, as representing a case of mild to moderate temporary amnesia. Much like transient global amnesia, which results in cognitive deficits similar to that of hippocampal amnesia but typically resolves with 24 h (Quinette et al., 2003, 2006), the amnesia-like effects of sleep deprivation are expected to resolve without long-term consequences. However, unlike transient global amnesia, the progression of sleep deprivation-induced amnesia is less severe and does not have a sudden and rapid onset. Before addressing the key question of whether such binding problems are manifest under sleep deprivation, we first must acknowledge an important caveat. Sleep deprivation produces some deficits in cognition that are different from problems experienced by patients with MTL lesions. For instance, sleep deprivation disrupts PFC functioning and connectivity with other brain regions (Yoo et al., 2007a), leading to problems directing attentional resources in pursuit of goals (Chee and Tan, 2010) and controlling emotional responses (Stenson et al., 2021). The most noteworthy example is the deficit in vigilant attention produced by sleep deprivation (Lim and Dinges, 2008; Basner and Dinges, 2011; Hudson et al., 2020). Amnesic patients do not typically have problems with vigilant attention, and if a memory task was administered to sleep-deprived subjects in a way that taxes vigilant attention, then lapses of attention could themselves produce a failure to encode stimuli. Nonetheless, in the tests of memory under sleep deprivation discussed below, task pacing and other means of ensuring that stimuli were processed, such as use of orienting tasks, reduce or eliminate lapses of vigilant attention as a potential source of memory deficits.

### 3.1. Binding deficits in explicit and implicit LTM

A comparison of the memory-based deficits observed in amnesia and sleep deprivation is included in Table 1. Like amnesia patients, sleep-deprived subjects show impaired learning and retention of new episodic information, such as lists of words or series of images (Drummond et al., 2000; Walker and Stickgold, 2006; Yoo et al., 2007b). In addition, sleep-deprived subjects show deficits in relational memory, with worse performance on tasks that require binding compared to their baseline performance or their rested counterparts (Harrison and Horne, 2000a; Tempesta et al., 2016; Ratcliff and Van Dongen, 2018; Kurinec et al., 2021). For example, Kurinec et al. (2021) presented subjects, assigned to either a sleep deprived or rested control condition, with a series of words spoken by either a male or female speaker. Compared to their rested counterparts and their own rested baseline, sleep-deprived subjects were worse at recognizing the words presented during study, consistent with previous work showing sleep deprivation impairments in new learning. However, even when sleep-deprived subjects correctly recognized the studied words, they were less able to recognize their associated speaker. Thus, even when sleep-deprived subjects have successfully encoded information, sleep deprivation results in additional impairments to the ability to bind that information with its context.

There is less of a history of overlapping research paradigms to compare the effects of sleep deprivation and MTL damage on implicit memory. We do know that much like amnesia patients, sleep-deprived subjects show intact priming (López-Zunini et al., 2014; Casey et al., 2016) and preserved performance on some implicit skills (McWhirter et al., 2015). The limited evidence available indicates that sleep-deprived subjects are similarly impaired on implicit memory tasks involving relational memory. For instance, compared to rested controls, sleep-deprived subjects show poorer implicit sequence learning during sleep loss (Heuer et al., 1998; Heuer and Klein, 2003). Implicit sequence learning requires individuals to learn new, higher-order associations, and the acquisition of these relations has been found to activate the MTL in healthy adult subjects, regardless of whether subjects later display awareness of the associations (Schendan et al., 2003). Still, given the limited research on implicit memory and sleep deprivation in general, this pattern of effects must be interpreted cautiously.

There has also been considerable interest in the sleep-related consolidation of motor memories (see King et al., 2017 for a review). Consistent with findings from the amnesia literature indicating a role for hippocampal binding in complex motor sequence learning, the data on sleep-related consolidation show that hippocampal-cortical connections are important when the task is dependent on complex spatial or abstract associations, or explicit memory. However, to our knowledge, there has yet to be a direct test of how sleep deprivation affects simple versus complex motor sequence learning. Adopting tasks used in the amnesia literature that show dissociations in amnesia patients based on the need for binding of relations in implicit or motor learning tasks could provide a strong test of the TASL framework.

### 3.2. Dissociation of STM and LTM

As noted above, the classic dissociation of STM and LTM in performance of amnesic patients is not as clear cut as once believed. Nevertheless, it is certainly the case that hippocampal lesions affect LTM without affecting typical measures of verbal STM, such as digit span. The same is true of subjects under sleep deprivation, as Tucker and colleagues showed that speed of searching verbal STM was unaffected by sleep deprivation (Tucker et al., 2010; García et al., 2021).

The picture is more complex if we look beyond verbal STM, and consider working memory, i.e., functions involved in the storage and manipulation of information in the focus of attention. Working memory can involve manipulation of verbal, semantic, visuospatial, and other types of representations (Baddeley, 1986; Rubin et al., 2017). The precise nature of deficits in working memory associated with amnesia is still being investigated, but as discussed above, there is considerable evidence for the involvement of the hippocampus in

TABLE 1 Comparison of preserved and impaired memory processes in amnesia and sleep deprivation.

| Process | Task | In amnesia | Under sleep deprivation |
|---|---|---|---|
| **Explicit long-term memory** | | | |
| Item memory | Encoding of new episodic information (e.g., words or pictures) | Poorer memory for words and pictures than their matched controls (Huppert and Piercy, 1977; Hirst et al., 1986) | Worse memory for words and pictures compared to rested controls or their own rested performance (Drummond et al., 2000; Yoo et al., 2007b) |
| Relational memory | Associative memory, or memory for the relations among sets of stimuli | Deficit in associative memory for the studied stimuli sets compared to matched controls (Giovanello et al., 2003), regardless of the type of association among the stimuli (Konkel et al., 2008) | Poorer associative and sequential memory for the studied stimuli sets compared to their rested counterparts and/or own performance at rested baseline (Tempesta et al., 2016; Ratcliff and Van Dongen, 2018) |
| | Source memory, or memory for individual stimuli (items) and the context (source) in which they were originally presented | Worse memory for items and their sources compared to controls (Shimamura and Squire, 1991; Gold et al., 2006) | Worse memory for individual items and their sources compared to rested controls and their own baseline performance (Kurinec et al., 2021) |
| **Implicit long-term memory** | | | |
| Item memory[1] | Unconscious memory for skills | Preserved mirror reading (Cohen and Squire, 1980), mirror drawing, and tactile maze learning (Milner et al., 1968) | Similar encoding of a texture discrimination task as their rested counterparts (McWhirter et al., 2015) |
| | Priming | Similar conceptual and repetition priming as control subjects (Haist et al., 1991; Levy et al., 2004) | Intact verbal priming compared to their rested baseline (López-Zunini et al., 2014; Casey et al., 2016) |
| Relational memory | Implicit sequence learning, or learning relationships that occur among series of stimuli | Less able to learn higher-order associations than controls (Curran, 1997) | Poorer learning than their rested counterparts (Heuer et al., 1998; Heuer and Klein, 2003) |
| **Short-term memory** | | | |
| Item memory | Maintaining individual stimuli in the focus of attention | Preserved ability to maintain information in memory (Baddeley and Warrington, 1970; Cave and Squire, 1992) | Preserved working memory scanning (Tucker et al., 2010; Whitney et al., 2015) |
| Relational memory | Maintaining associations in the focus of attention for short periods of time | Impaired at detecting changes in item-location associations (Yee et al., 2014) and at maintaining object-location associations (Olson et al., 2006) | Poorer performance when spatial positions or color-location pairings must be maintained in memory (Chee and Chuah, 2007; Wee et al., 2013) |
| **Decision-making** | | | |
| Binding independent | Delay discounting, or choosing between smaller immediate rewards or larger, delayed rewards | Show similar discounting functions as matched controls (Kwan et al., 2012, 2013) | Show similar discounting functions as their rested baseline or rested counterparts (Acheson et al., 2007; Libedinsky et al., 2013) |
| Binding-dependent | Iowa Gambling Task (IGT), which requires subjects to learn to prefer the decks that result in long-term gains | Fail to learn to prefer the advantageous decks over time (Gutbrod et al., 2006; Gupta et al., 2009) | Do not learn to prefer the advantageous decks compared to their rested baseline (Killgore et al., 2006) |

[1]Although item memory can generally be conceived as a memory representation for a given stimulus, implicit item memory is better thought of as the "functional unit of repetition in a task" (Cohen et al., 1997, p. 150).

working memory processing when binding is needed, particularly when complex spatial relationships among objects must be processed (see Yonelinas, 2013), or when novel rather than familiar stimuli must be maintained (Rose et al., 2012). Consistent with the TASL framework, Chee and Chuah (2007) found a sleep deprivation-induced deficit in performance of a working memory task that required maintenance of the spatial positions of visual stimuli. Likewise, sleep-deprived subjects are impaired on tasks that require them to bind color-location pairings in working memory (Wee et al., 2013).

A working memory issue that has received more attention in the sleep literature than in research on amnesia is working memory updating (*cf.*, Spiers et al., 2001; Choo et al., 2005; Lythe et al., 2012). In many kinds of complex tasks, information must be continuously

moved into and out of the focus of attention, and this process is often studied in the laboratory using the N-back task (Jonides et al., 1997; Pelegrina et al., 2015). Subjects performing the N-back task see a series of stimuli, often letters, and must report whether the stimulus on the current trial is the same as one N (typically from 1 to 3 items) back. Sleep-deprived subjects are impaired on the N-back task compared to their rested state (Choo et al., 2005). Relevant to the TASL framework is evidence that hippocampal-PFC pathways are invoked in the N-back task, which is consistent with the known role of the hippocampus in maintaining temporal order information (Costers et al., 2020). However, it is misleading to think of the N-back task as a simple measure of working memory updating because multiple cognitive processes are needed for successful performance including inhibition of distractors, and multiple interactions among the PFC, hippocampus, and basal ganglia are involved (Rac-Lubashevsky and Kessler, 2016; Costers et al., 2020). Hence, there are several potential sources of performance decrements on the N-back. More research is needed to determine whether the effects of sleep deprivation on the N-back are based on compromised hippocampal functioning, or due to other contributors to task performance.

Of course, sleep deprivation effects on cognition have often been attributed to deficits in PFC functioning and the circuits connecting the PFC to striatal and parietal areas (Chee et al., 2010; Wickens et al., 2015; Krause et al., 2017). The data on when STM and working memory tasks are, and are not, compromised by amnesia suggest that the need for binding in working memory tasks may well predict the degree of performance impairment from sleep deprivation, and serve as a further test of the TASL framework.

### 3.3. Binding in other cognitive domains

Studies of the effect of sleep deprivation on logical reasoning and complex decision-making have typically concluded that sleep deprivation has minimal effects on these cognitive processes (Harrison and Horne, 2000b; Killgore, 2010; Lim and Dinges, 2010). Similarly, preserved performance in these domains by people with amnesia was an important reason that amnesia was considered to be a specific deficit in declarative memory. But as we noted earlier, decision-making tasks such as the IGT, that require binding novel associations based on decision outcomes, are disrupted by MTL damage. A similar pattern of effects is observed with sleep deprivation during the IGT. Sleep-deprived subjects are less able to learn to choose from the advantageous decks on the IGT, resulting in poorer decision-making performance compared to controls (Killgore et al., 2006; Gupta et al., 2009).

The use of choice outcome feedback to guide future actions is a key component of adaptive behavior in everyday life, and it can mean the difference between success and disaster when making decisions in high stakes medical, military, and first responder settings. Consistent with the TASL framework, difficulty with binding choices and outcomes to guide future actions is not limited to the IGT. In amnesia patients and people deprived of sleep, impaired use of choice outcome feedback to guide subsequent choices has been demonstrated in decisions involving risk and in tasks requiring cognitive flexibility (Brand et al., 2009; Foerde et al., 2013; Whitney et al., 2015; Honn et al., 2019).

## 4. Conclusions and implications

Based on the accumulating evidence that the functioning of the hippocampus and associated MTL areas are disrupted by sleep deprivation in humans and in animal models (Tudor et al., 2016; Guttensen et al., 2023), it would be surprising if we did not observe significant effects of sleep loss on memory. However, to understand what aspects of memory are, and are not, affected by sleep deprivation requires a deeper understanding of the role of the hippocampus in memory. The TASL framework draws on the amnesia literature and the role of the hippocampus in binding to provide an organizing perspective on sleep deprivation and memory, and on how sleep loss is likely to affect other cognitive tasks that depend on binding.

The existing literature on sleep deprivation deficits in memory provides substantial support for the TASL framework. Although sleep deprivation does not lead to the same degree of impairment typically seen in amnesia resulting from MTL lesions, the pattern of impaired and preserved memory functioning observed in sleep-deprived subjects is remarkably consistent with that characteristically seen in amnesia patients. This is true not only for explicit recall and recognition tasks, but also for relational memory of items with their context. Even in the case of implicit memory, the pattern of preserved functions and deficits appears similar in amnesic patients and people who have had a night of sleep deprivation.

The TASL framework also calls attention to the possibility that some sleep deprivation effects outside of LTM may result from problems with relational binding. Amnesia patients show preserved STM maintenance along with deficits in working memory tasks that require processing of temporal, spatial, and other relational information, and this pattern has been observed after sleep loss as well.

The similarities between the effects of sleep deprivation and amnesia on various memorial processes lead to specific predictions that would test this framework. To begin, there remain several aspects of memory that have yet to be extensively or explicitly investigated under sleep deprivation, such as dissociating between explicit and implicit memories, or dissociating between tests that differ in their need for hippocampal involvement. From the TASL framework, we expect that sleep-deprived subjects would show similar patterns of performance as amnesia patients, insofar as the task is largely dependent on the hippocampus. Such a deliberate comparison would not only test the framework but would also provide clarity on how sleep deprivation and amnesia effects on memory differ. Separately, given that amnesia is characterized as a deficit in relational memory (Ryan et al., 2000), the TASL framework implies that sleep deprivation should result in impairment on all tasks that require binding, including those that do not fall under the memory domain. Such a prediction has wide-reaching implications with the obvious importance of binding to the development of schemas, inferences, and other forms of abstract representations that support flexible decision-making (Biderman et al., 2020; Vaidya and Badre, 2022). Further, because sleep deprivation does not result in deficits to the same extent as amnesia, we expect that any binding that does occur should result in less precise representations, as these similarly depend on intact hippocampal functioning (Ekstrom and Yonelinas, 2020). On a positive note, the TASL framework implies that some of the strategies that promote memory and performance

in amnesia patients should also benefit sleep-deprived individuals. For example, unitizing or integrating separate representations into a single unit (e.g., creating the novel compound word "cloudlawn" to remember the words "cloud" and "lawn" together) has been shown to improve relational memory for certain types of amnesia (Quamme et al., 2007; Ryan et al., 2013). Testing these predictions will determine the extent to which the TASL framework is useful for guiding future research on sleep deprivation and memory-related processes.

One of the most important implications of the TASL framework is that while considerable research has documented sleep deprivation effects on LTM, disruptions in hippocampal functioning are expected to have specific consequences for relational processing needed on shorter timescales such as working memory processing, and on some kinds of decision-making tasks. In addition, the data we have reviewed here strongly suggest that to understand dissociations in the effects of sleep deprivation on cognitive performance, it will be important to not only examine what cognitive processes are needed for a given task, but also the *representations* on which the processes operate. The hippocampus and PFC-hippocampus interactions support multiple cognitive operations, but the contribution of this circuitry depends in part on the nature of the information being represented (Rubin et al., 2017). For example, as noted earlier, working memory is typically thought of as PFC driven processing, but the hippocampus is crucial when the information in the focus of attentional depends on maintenance of information about temporal or spatial relationships among elements.

Clearly, sleep loss disrupts more than the cognitive operation of binding, so the TASL framework can only serve as a guide to one source of impairment in cognitive performance. Nevertheless, future studies of the effects of sleep deprivation that examine whether it produces similar deficits, and islands of preserved performance, as found in the performance of amnesia patients on implicit memory, working memory, and decision-making, could help isolate the role of hippocampal disruption apart from other consequences of sleep loss.

## Author contributions

PW and JH contributed to the conception of the review. PW organized the structure of the manuscript. PW and CK wrote the original draft of the manuscript. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

The authors declare that this research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



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# Recovery sleep attenuates impairments in working memory following total sleep deprivation

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**Introduction:** The detrimental effects of sleep deprivation (SD) on cognitive function and quality of life are well known, and sleep disturbances are a major physical and mental health issue worldwide. Working memory plays an important role in many complex cognitive processes. Therefore, it is necessary to identify strategies that can effectively counteract the negative effects of SD on working memory.

**Methods:** In the present study, we utilized event-related potentials (ERPs) to investigate the restorative effects of 8h of recovery sleep (RS) on working memory impairments induced by total sleep deprivation for 36h. We analyzed ERP data from 42 healthy male participants who were randomly assigned to two groups. The nocturnal sleep (NS) group completed a 2-back working memory task before and after normal sleep for 8h. The sleep deprivation (SD) group completed a 2-back working memory task before and after 36h of total sleep deprivation (TSD) and after 8h of RS. Electroencephalographic data were recorded during each task.

**Results:** The N2 and P3 components—which are related to working memory—exhibited low-amplitude and slow-wave characteristics after 36h of TSD. Additionally, we observed a significant decrease in N2 latency after 8h of RS. RS also induced significant increases in the amplitude of the P3 component and in the behavioral indicators.

**Discussion:** Overall, 8 h of RS attenuated the decrease in working memory performance caused by 36 h of TSD. However, the effects of RS appear to be limited.

KEYWORDS

**sleep deprivation, recovery sleep, working memory, event-related potential, N2, P3**

## 1. Introduction

Proper sleep has been shown to exert beneficial effects on memory, cognitive function, work performance, and immune-related parameters (Walker and Stickgold, 2004; Stickgold, 2005; Anderson and Horne, 2006; Djonlagic et al., 2009). However, the incidence of sleep-related problems continues to increase. While the most direct and obvious behavioral manifestation of sleep deprivation (SD) is drowsiness, it also significantly impairs cognitive function (Drummond et al., 2006; Killgore et al., 2006; Alhola and Polo-Kantola, 2007; Anderson and Platten, 2011). Honn et al. (2020) demonstrated that SD reduces processing speed in visual search, spatial memory, paired associative learning, motor response, and other cognitive tasks. Additional evidence suggests that SD significantly increases response times in working memory tasks. Such changes are also accompanied by decreased activation of the frontoparietal cortex (FPC), which

plays an important role in cognitive control. Specifically, the FPC can bypass top-down cognitive control, thus enabling individuals to focus on goal-related information while suppressing irrelevant information (Smallwood et al., 2012; Wen et al., 2013).

Working memory is a limited-capacity system involved in the temporary storage and maintenance of information related to a specific task (Baddeley, 2010). As such, it acts as a bridge between short- and long-term memory (Baddeley, 2000) and is involved in operating, processing, and executing various control processes. Previous studies have consistently demonstrated that SD significantly impairs working memory (Lo et al., 2012; Gerhardsson et al., 2019). In addition to decreasing the quality of information stored in the working memory, SD reduces processing speeds and alters event-related potentials (ERPs) during task performance by prolonging latency and reducing the amplitude of the N2 and P3 components (Zhang et al., 2019). These changes are also associated with a decreased ability to discriminate between target stimuli (Koslowsky and Babkoff, 1992), reduced availability of disposable attentional resources, and alterations in selective attention toward emotional stimuli/signal processing (Schacht et al., 2008).

Numerous research groups have aimed to identify interventions that can effectively counteract the aforementioned negative consequences of SD. Although caffeine and other drugs can effectively maintain work performance and alertness (Rosenthal et al., 1991; Rogers et al., 2005; Biggs et al., 2007), they have limited effects on high-level cognitive functions. In addition, the use of caffeine may be associated with recovery costs in individuals with long-term sleep deficiency (Doty et al., 2017). Recovery sleep (RS) refers to a short period of adequate sleep following SD, and it represents a potential non-pharmacological strategy for combating the effects of SD on cognitive function. Some studies have reported that RS can attenuate SD-induced hyperalgesia (Roehrs et al., 2012; Stroemel-Scheder et al., 2020) and cognitive impairment (Ruggiero and Redeker, 2014). RS also alleviates fatigue and improves attention/alertness, and longer periods of RS are associated with the restoration of cognitive function to a greater degree (Studte et al., 2015). One study reported that 8 h of RS can attenuate impairments in response inhibition caused by 36 h of total sleep deprivation (TSD) (Jin et al., 2015). One night of TSD causes obvious changes in the topological characteristics of the small-world network in the brain. Although two nights of RS can completely restore the global properties of the brain network, it has not been found to induce changes in the local function (Jiang et al., 2018). Following 58 h of wakefulness, sleep stage distribution resembles that of a baseline night when the sleep duration of the recovery night is extended to 14 h (Hennecke et al., 2019). Moreover, a 90-min nap during a day of sleep deprivation can restore hippocampus-dependent learning, and the structural composition of the hippocampus has been shown to predict the success of learning recovery (Saletin et al., 2016). Nevertheless, one or two nights of RS after complete or chronic sleep loss cannot sufficiently attenuate the associated neurobehavioral deficits or restore self-monitoring abilities and brain metabolism (Banks et al., 2010; Lo et al., 2016; Boardman et al., 2018). Therefore, the amount of sleep required to restore cognitive function following extended wakefulness remains unclear.

Impairments in cognitive function due to SD inevitably affect the task performance of individuals. Previous studies have used both neurophysiological and behavioral indicators to assess an individual's physiological and psychological states. Among these, neurophysiological indicators are more sensitive for assessing the effects of SD. Chai et al. (2020) reported that two nights of RS after one night of TSD restored hippocampal connectivity to normal levels but did not fully restore behavioral performance or its associations with hippocampal connectivity (Chai et al., 2020). A study investigating joint rhythm (Gumenyuk et al., 2014) reported that significant differences in the reorienting negativity amplitude (related to behavioral responses) could be explained by the increased sensitivity of neurophysiological indicators. Electroencephalographic (EEG) data recorded from the human scalp reflect potential changes that indicate the activity of the brain, and ERPs are special evoked potentials related to endogenous neural activity and cognitive function. After RS, information processing efficiency was also restored which related to the promotion and recovery of memory by sleep. The N2 component of the ERP reflects performance monitoring and cognitive control (top-down and bottom-up), and its neural source has most consistently been identified as the anterior midcingulate cortex (aMCC) (Folstein and Van Petten, 2008; Huster et al., 2010). Some researchers have suggested that the P300 component plays a crucial role in brain processing at the intersection between perception and decision making (Verleger et al., 2005). However, others have suggested that it is more commonly involved in synthesizing and carrying information related to conscious access, attentional moderation, and post-response adaptations (Polich, 2007; Dehaene and Changeux, 2011; Raud and Huster, 2017). Previous studies have also demonstrated that the P3 wave is related to the renewal of working memory content and it decreases with an increase in working memory load (Donchin and Fabiani, 1991; Peng et al., 2020). Therefore, N2-P3 components that relate to different information processes will also change after RS (Zhang et al., 2019). Sleep cycles include four stages. Healthy adults need to sleep for 4–5 cycles every night, which typically requires approximately 8 h (Malik et al., 2018). Sleep "reshapes" hippocampal synapses, making room for learning the next day (Spano et al., 2019).

At present, it remains to be determined whether 8 h of RS can attenuate SD-induced impairments in working memory. In the present study, we investigated the potential restorative effects of RS on working memory and ERP latency/amplitude after 36 h of TSD. We tested the following hypotheses: (a) 8 h of RS partially attenuates the deleterious effects of 36 h of TSD on working memory in our participants and (b) following 36 h of TSD, 8 h of RS reduces the latencies of the working memory-related N2-P3 components and increases their amplitudes. A lack of significant differences between 36 h TSD and subsequent 8 h RS would suggest that the restorative effects of 8 h of RS are limited.

## 2. Materials and methods

### 2.1. Participants

The present study included 42 healthy male college students (mean age, 23 years; age range, 21–28 years). Participants were randomly assigned to two groups: the nocturnal sleep (NS) group (20 participants) and the SD group (22 participants). All participants were right-handed and maintained good sleep habits (Pittsburgh Sleep Quality Index <5 points) (Buysse et al., 1989). None of the patients reported a history of mental or physical illnesses, and none had previously undergone psychophysiological testing. All participants

had normal or corrected-to-normal visual acuity, and their IQ scores were greater than the population average (Raven test scores >110 points). The research staff provided a full explanation of the study procedures prior to the experiment. All participants were asked to refrain from smoking, drinking alcohol/coffee, and taking any drugs for at least 48 h prior to the experiment, and were instructed to maintain a normal sleep pattern for 1 week. All participants slept for 7–9 h per day between 10:00 pm and 9:00 am, and their sleep routines throughout the study period were confirmed using sleep diaries. All participants provided written informed consent and received monetary compensation upon the completion of the experiment. This study was approved by the Ethics Committee of the Fourth Military Medical University.

## 2.2. Experimental design

In the present study, we used three tasks: a 2-back pronunciation working memory (PWM) task (Figure 1A), a 2-back spatial working memory (SWM) task (Figure 1B), and a 2-back object working memory (OWM) task (Figure 1C). The stimuli for these tasks included 15 case-insensitive English letters (excluding similar letters), small black squares, and 12 geometric figures. Each task lasted approximately 5 min and included 122 trials. Target stimuli were presented for 400 ms, with an inter-stimulus interval of 1,600 ms. Participants were asked to match the current stimulus with the stimulus presented two trials earlier, and were instructed to press the left mouse button for matching stimuli and the right mouse button for mismatching stimuli. The matching and mismatching stimuli were presented in a pseudo-random order in a 1:1 ratio.

According to the kind of stimulus material, working memory can be divided into the phonetic, spatial, and object types. To perform a more thorough investigation than past publications on the effects of sleep deprivation and restorative sleep on working memory, this study examined three types of working memory tasks. The tasks were independent of each other, and the participants exercised to eliminate the connection effect before engaging in the formal experiment.

## 2.3. Experimental procedures

A mixed experimental design was used. Prior to testing, all participants practiced the experimental tasks until they reached an accuracy of >90% (to exclude the influence of practice). All the participants visited the laboratory once. The NS group arrived at the laboratory at 6:00 p.m. on the day of the experiment and performed the first task at 8:00 p.m. (baseline state; NS-BS). Following this, the participants slept in the laboratory that night (ensuring a sleep time of at least 8 h), woke up the next day at 7:00 am, and performed the second task at 8:00 am (0 h sleep deprivation; NS-SD0) with EEG data being recorded simultaneously (Figure 2). The SD group slept in the laboratory the night prior to completing the experimental tasks, and all participants were instructed to sleep from 11:00 pm to 7:00 am to ensure a sleep time of at least 8 h. The SD group underwent 36 h of SD followed by 8 h of RS (Figure 3). TSD was initiated at 8:00 am the following morning. The 2-back working memory tasks were performed both before and after TSD and 8 h after RS, with EEG data being recorded simultaneously.

Sleep inertia can be observed after waking up from complete and habitual night sleep and seems to be a common step in the sleep–wake transition process. Sleep inertia tends to be exacerbated by prior sleep loss or extended wakefulness prior to a sleep episode (Miccoli et al., 2008; Hilditch et al., 2017). To avoid sleep inertia, RS was implemented from 11:00 p.m. to 7:00 a.m., and a third set of EEG data was acquired at 8:00 a.m. on the third day (Dinges, 1990). Previous research has demonstrated that individual performance is

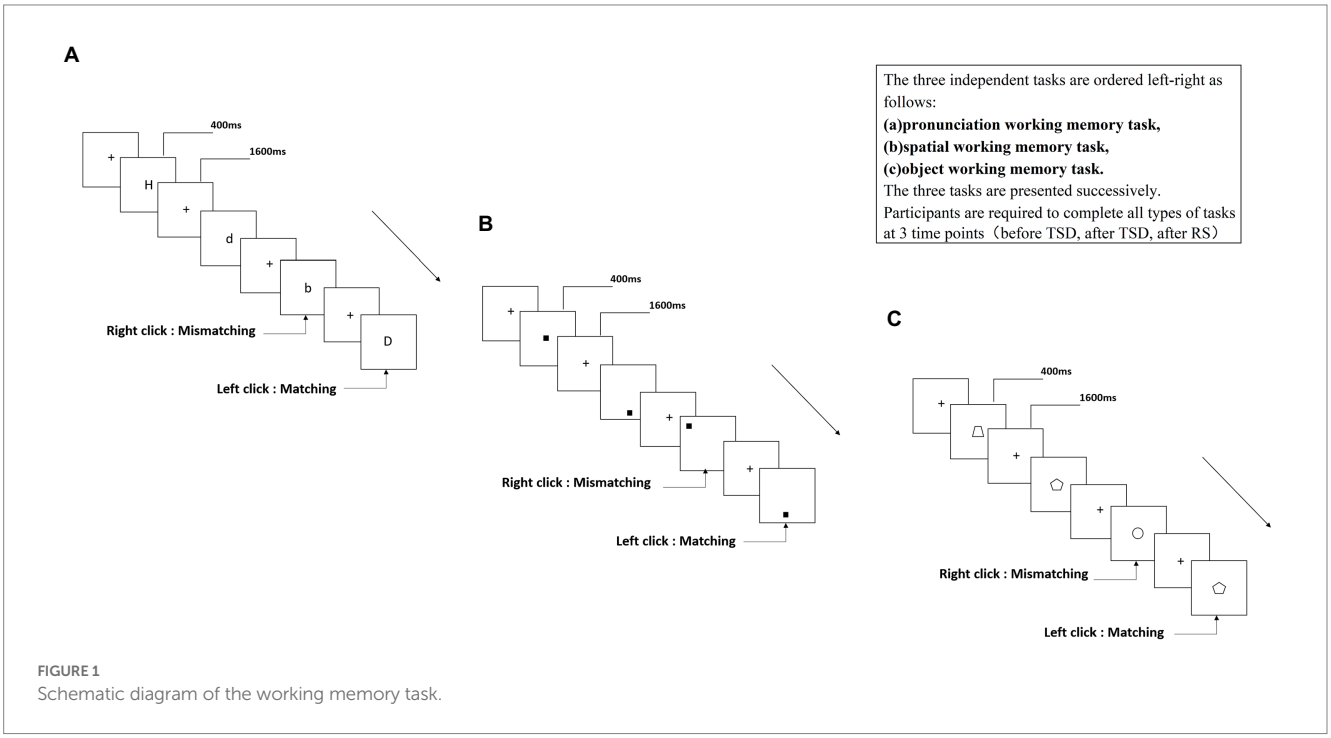


FIGURE 1
Schematic diagram of the working memory task.

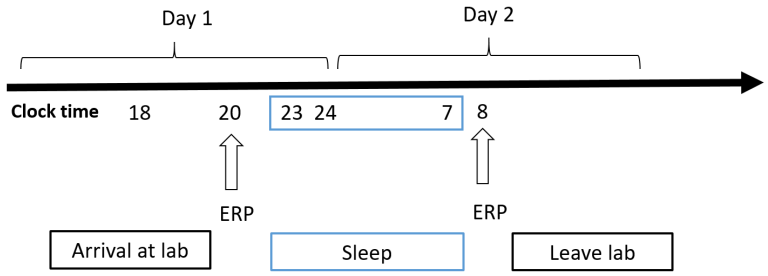


FIGURE 2
Experimental design for the nocturnal sleep (NS) group. Participants completed the tasks twice: before and after 8h of sleep in the laboratory. Electroencephalographic data were recorded simultaneously. The arrows indicate various time points during the 2-back working memory task.

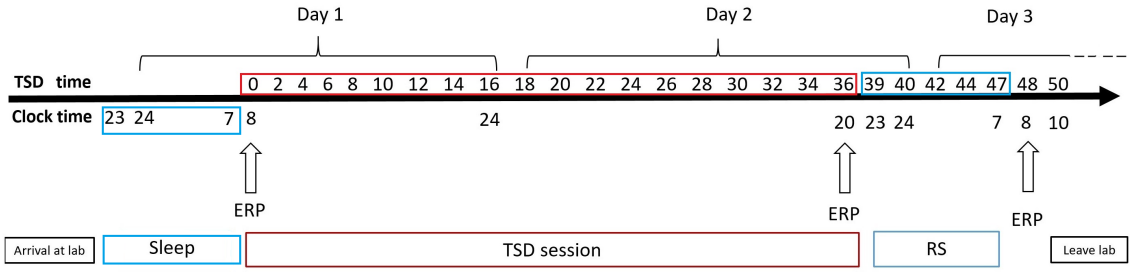


FIGURE 3
Experimental design for the sleep deprivation (SD) group. After 8h of sleep in our laboratory, participants underwent 36h of total sleep deprivation (TSD), followed by 8h of recovery sleep (RS). Electroencephalographic data were recorded simultaneously. The arrows indicate various time points during the 2-back working memory task.

relatively impaired within 5 min of awakening and gradually returns to normal over a period of 15–30 min (Dinges, 1986). Before TSD and 8 h after RS, the three types of working memory tasks were performed from 8:00 a.m. to 8:30 a.m. After TSD, the three types of working memory tasks were performed from 20:00 to 20:30. Two participants completed the experiment simultaneously. Two medical workers and one researcher were present throughout the TSD period to prevent the participants from sleeping or napping. During the experiment, participants were allowed to eat, drink, and perform light physical activities, but were not allowed to engage in strenuous exercise or ingest caffeine, alcohol, or tea. The illumination in the laboratory was set to 100 lx with a normal fluorescent lamp.

## 2.4. EEG recordings

EEG data were acquired in a dark, sound-proof, and electronically shielded EEG laboratory using 64-electrode caps. Stimuli were generated and presented using the Stim-2 software (NeuroScan Inc., United States). The electrodes were arranged in accordance with the international 10–20 system. Horizontal and vertical electrooculograms were recorded during EEG acquisition, and the bilateral mastoid process was used as the reference electrode. The recordings were performed at 1,000 Hz, and the channel impedance was maintained at below 5 kΩ.

## 2.5. Data analysis

We analyzed various behavioral parameters, including mean reaction time, accuracy, and the number of correct responses per unit time (number of correct responses per unit time = correct ratio × 1,000/correct time).

In this study, the EEG amplitude represented brain potential intensities. The amplitude size is closely related to the number of neurons involved in synchronous firing, as well as the arrangement direction of the neurons. Latency provided a measure the time interval from stimulus presentation to the peak amplitude value for each condition (Kiesel et al., 2008). ERP data could not be recorded in three cases due to technical issues. Data from these three participants were excluded during post-processing (NS group: 19; SD group: 20).

EEG data were pre-processed using the SCAN 4.3 software (Neuroscan, Inc., United States), following which ocular artifacts were removed *via* regression analysis. The data were bandpass filtered at 0.05–30 Hz (frequency slope: 24 dB/oct) and were divided into 900-ms epochs (−100 ms to 800 ms). A period of 100 ms prior to stimulation was included for baseline correction. Trials in which the voltage exceeded ±100 μV were excluded. Non-physiological artifacts mainly included those generated from the contact between the electrode and the scalp to the device or to the environment (i.e., the environment around the device or the device in the participant). Generally, non-physiological artifacts display various waveforms, which may preclude data interpretation in severe cases (Maddirala and Shaik,

2016). The mean number of accepted trials was 92.4 ± 17.46 (NS: 95.6 ± 7.85 vs. SD: 89.2 ± 27.07, $p > 0.05$). As guided by previous studies, P3 (250–450 ms) and N2 components (150–350 ms) were analyzed for the following channels: F3, Fz, F4, C3, Cz, C4, P3, Pz, and P4 (Zhang et al., 2019; Peng et al., 2020).

Repeated-measures analysis of variance (ANOVA) was used to analyze both the behavioral data and ERP findings using SPSS (version 22.0, IBM Corp., United States).

## 2.6. Statistical analysis of data from the NS and SD groups

For ERP analyses, we assessed the main and interaction effects of the groups (NS and SD), sleep states (NS-BS and NS-SD0; SD-SD0 and SD-SD36), tasks (PWM, SWM, and OWM), regions (frontal, central, and parietal), and sites (left, middle, and right). Behavioral data were compared between the two groups (NS and RS), two sleep states (no-sleep deprived: NS-BS and NS-SD0; sleep deprived: SD-SD0 and SD-SD36), and three tasks (PWM, SWM, and OWM). Greenhouse–Geisser corrections for non-sphericity and *post hoc* tests with Bonferroni correction were performed. The results are presented as the mean and standard deviation.

## 2.7. Statistical analysis of data from the SD groups

To analyze ERP data, we assessed the main and interaction effects of sleep states (SD-SD0, SD-SD36, and RS-8 h), tasks (PWM, SWM, and OWM), regions (frontal, central, and parietal), and sites (left, middle, and right). We analyzed the same behavioral parameters as those mentioned above, and compared behavioral data between the three sleep states (RS-SD0, RS-SD36, and RS-8 h) and three tasks (PWM, SWM, and OWM). Greenhouse–Geisser corrections for non-sphericity and *post hoc* tests with Bonferroni correction were performed. The results are presented as the mean and standard deviation.

# 3. Results

## 3.1. Results of NS vs. SD comparisons

### 3.1.1. Behavioral performance

The mean reaction time, accuracy, and number of correct responses per unit time are presented in Tables 1, 2. For accuracy ($F_{(1,\ 37)} = 16.420$, $p < 0.001$, $\eta^2_p = 0.307$) and number of correct responses per unit time ($F_{(1,\ 37)} = 4.869$, $p = 0.034$, $\eta^2_p = 0.116$), there were significant interaction effects between group and sleep state. These results suggested that in the SD group, accuracy ($p < 0.001$) and the number of correct responses per unit of time ($p = 0.009$) decreased significantly after 36 h of TSD. However, there was no statistically significant differences between the two states in the NS group (Figure 4). No other main or interaction effects were statistically significant.

### 3.1.2. The N2 component

The descriptive statistics for the N2 component of the NS group are presented in Tables 3, 4. N2 latency ($F_{(1,\ 37)} = 4.426$, $p = 0.042$, $\eta^2_p = 0.107$) was affected by significant interaction effects between group and sleep state. N2 latency was significantly prolonged after a 36 h TSD in the SD group ($p = 0.002$). However, there was no difference in N2 latency between the two sleep states in the NS group ($p = 0.085$). For N2 amplitude, there were no significant interaction effects between the groups and sleep states ($F_{(1,\ 37)} = 0.004$, $p = 0.947$, $\eta^2_p < 0.001$). No other main or interaction effects were statistically significant.

TABLE 1 Behavioral performance (mean±standard deviation) in the three types of 2-back tasks in the sleep deprivation (SD) group.

| | SD-0h | | | SD-36h | | | RS-8h | | |
|---|---|---|---|---|---|---|---|---|---|
| | PWM | SWM | OWM | PWM | SWM | OWM | PWM | SWM | OWM |
| Mean reaction time (ms) | 539.94 (101.20) | 520.16 (91.58) | 522.19 (88.44) | 561.88 (104.23) | 527.46 (94.15) | 543.85 (107.88) | 528.09 (66.34) | 498.80 (84.98) | 526.07 (80.19) |
| Correct rate (%) | 0.89 (0.10) | 0.95 (0.04) | 0.88 (0.09) | 0.82 (0.13) | 0.85 (0.12) | 0.79 (0.13) | 0.88 (0.08) | 0.93 (0.04) | 0.87 (0.07) |
| Correct number/s | 1.73 (0.45) | 1.88 (0.35) | 1.76 (0.44) | 1.53 (0.40) | 1.68 (0.41) | 1.53 (0.45) | 1.70 (0.30) | 1.93 (0.36) | 1.69 (0.34) |

PWM, pronunciation working memory; SWM, spatial working memory; OWM, object working memory.

TABLE 2 Behavioral performance (mean±standard deviation) in the three types of 2-back tasks in the nocturnal sleep (NS) group.

| | BS | | | SD-0h | | |
|---|---|---|---|---|---|---|
| | PWM | SWM | OWM | PWM | SWM | OWM |
| Mean reaction time (ms) | 562.51 (163.77) | 518.58 (137.09) | 519.83 (117.88) | 557.28 (165.27) | 495.90 (136.18) | 538.57 (134.62) |
| Correct rate (%) | 0.90 (0.10) | 0.93 (0.05) | 0.90 (0.07) | 0.90 (0.08) | 0.94 (0.04) | 0.90 (0.07) |
| Correct number/s | 1.74 (0.53) | 1.92 (0.50) | 1.83 (0.48) | 1.76 (0.54) | 2.04 (0.55) | 1.79 (0.48) |

PWM, pronunciation working memory; SWM, spatial working memory; OWM, object working memory.

### 3.1.3. The P3 component

The descriptive statistics for the P3 component of the NS group are presented in Tables 3, 4. For P3 latency ($F_{(1, 37)} = 0.002$, $p = 0.962$, $\eta^2_p < 0.001$) and P3 amplitude ($F_{(1, 37)} = 0.902$, $p = 0.348$, $\eta^2_p = 0.024$), there were no significant interaction effects between group and sleep state. However, a simple effect analysis revealed that before sleep deprivation, the P3 amplitude was significantly lower in the SD group ($p = 0.013$). The average amplitudes and latencies of P3 as elicited by the nine electrode sites are presented in Figures 5, 6. No other main or interaction effects were statistically significant.

## 3.2. Results of SD vs. RS comparisons

### 3.2.1. Behavioral performance

The results of the behavioral experiments are presented in Table 1. The mean reaction time tended to be longer after the 36 h TSD and tended to be shorter after 8 h of RS, although the differences were not significant ($F_{(2, 38)} = 1.07$, $p = 0.353$, $\eta^2_p = 0.053$). For accuracy ($F_{(2, 38)} = 17.023$, $p < 0.001$, $\eta^2_p = 0.473$) and the number of correct responses per unit time ($F_{(2, 38)} = 5.893$, $p = 0.006$, $\eta^2_p = 0.237$), there was a significant main effect of sleep state. The accuracy and number of correct responses per unit time were significantly decreased after the 36 h TSD (accuracy: $p < 0.001$; number of correct responses per unit time: $p = 0.02$) and were restored after the 8 h RS (accuracy: $p < 0.001$; number of correct responses per unit time: $p = 0.006$) (Figure 7). No other main or interaction effects were statistically significant.

### 3.2.2. The N2 component

The descriptive statistics for the N2 component of the RS group are presented in Tables 5, 6. For the N2 latency, there was a significant main effect of sleep state ($F_{(2, 38)} = 4.511$, $p = 0.017$, $\eta^2_p = 0.192$). After the 36 h TSD, the N2 latency ($p = 0.01$) increased significantly and showed a decreasing trend after the 8 h RS ($p = 0.11$). There was no main effect of sleep state on the amplitude of N2 ($F_{(2, 38)} = 0.465$, $p = 0.631$, $\eta^2_p = 0.024$). No other main or interaction effects were statistically significant.

### 3.2.3. The P3 component

The descriptive statistics for the P3 component of the RS group are presented in Tables 5, 6. For P3 amplitude, there was a significant main effect of sleep state ($F_{(1.52, 28.95)} = 4.948$, $p = 0.012$, $\eta^2_p = 0.207$). After the 36 h TSD, the P3 amplitude ($p = 0.003$) decreased significantly and was restored after the 8 h RS ($p = 0.015$). We also observed a significant

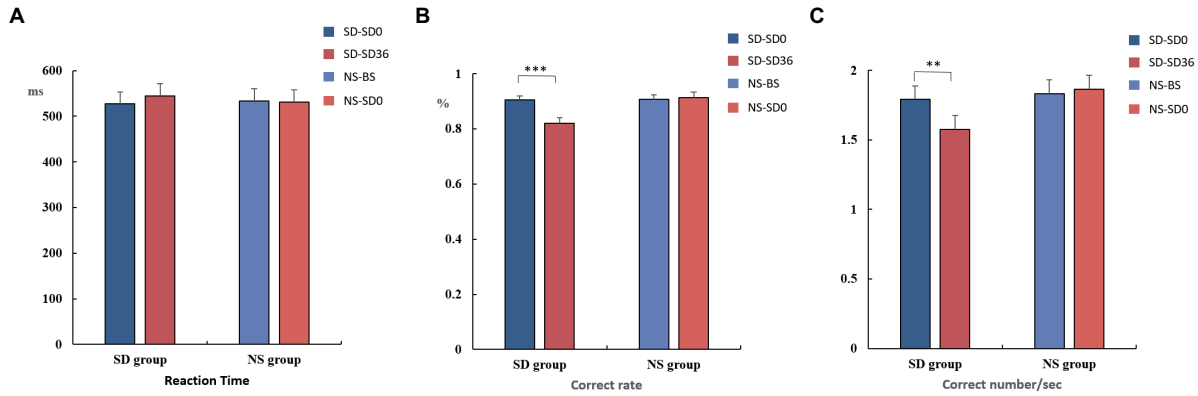


FIGURE 4
Reaction time, rate of correct responses, and number of correct responses per unit time (mean±standard deviation). SD, sleep deprivation; BS, baseline; NS, nocturnal sleep. *$p$<0.05, **$p$<0.01, ***$p$<0.001.

TABLE 3 Grand-average peak latency of the N2 and P3 components for correct responses across multiple electrode sites at baseline and after 0h of sleep deprivation (SD0) in the nocturnal sleep (NS) group.

| | | Baseline | | SD-0h | |
|---|---|---|---|---|---|
| | | N2 | P3 | N2 | P3 |
| F3 | M (SD) | 238.94 (29.63) | 363.22 (33.47) | 241.55 (32.17) | 363.44 (30.42) |
| Fz | M (SD) | 237.69 (29.24) | 359.19 (33.29) | 242.07 (31.22) | 358.87 (33.56) |
| F4 | M (SD) | 232.58 (27.75) | 360.64 (31.81) | 230.14 (28.15) | 359.71 (32.25) |
| C3 | M (SD) | 228.34 (26.27) | 353.49 (31.16) | 228.347 (30.14) | 358.41 (32.03) |
| Cz | M (SD) | 222.83 (23.89) | 351.83 (30.23) | 227.66 (27.92) | 350.44 (32.31) |
| C4 | M (SD) | 220.66 (24.04) | 359.01 (28.57) | 218.66 (26.21) | 353.82 (29.44) |
| P3 | M (SD) | 206.81 (31.96) | 346.72 (28.67) | 207.25 (29.44) | 347.09 (38.32) |
| Pz | M (SD) | 207.52 (23.71) | 345.30 (31.13) | 214.69 (29.20) | 347.03 (36.55) |
| P4 | M (SD) | 212.61 (32.96) | 322.81 (31.84) | 205.50 (33.84) | 332.04 (32.02) |

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TABLE 4 Grand-average peak amplitude of the N2 and P3 components for correct responses across multiple electrode sites at baseline and after 0h of sleep deprivation (SD0) in the nocturnal sleep (NS) group.

| | | Baseline | | SD-0h | |
|---|---|---|---|---|---|
| | | N2 | P3 | N2 | P3 |
| F3 | M (SD) | −2.90 (3.94) | 6.97 (2.37) | −2.11 (3.80) | 5.76 (2.89) |
| Fz | M (SD) | −3.22 (4.40) | 7.79 (2.36) | −2.22 (3.97) | 6.63 (3.48) |
| F4 | M (SD) | −2.45 (4.05) | 8.13 (2.60) | −1.54 (3.75) | 7.40 (3.24) |
| C3 | M (SD) | −2.04 (3.61) | 7.73 (2.06) | −1.94 (2.86) | 6.91 (2.97) |
| Cz | M (SD) | −1.66 (4.24) | 9.38 (2.26) | −1.95 (3.52) | 8.02 (3.41) |
| C4 | M (SD) | −1.02 (3.50) | 9.30 (2.34) | −1.45 (3.13) | 8.17 (3.26) |
| P3 | M (SD) | −2.89 (5.50) | 7.76 (2.49) | −2.20 (4.84) | 7.80 (3.01) |
| Pz | M (SD) | −1.08 (3.59) | 8.51 (2.68) | −1.76 (3.63) | 8.21 (2.73) |
| P4 | M (SD) | −1.17 (4.00) | 7.02 (2.43) | −2.34 (5.35) | 7.74 (3.07) |

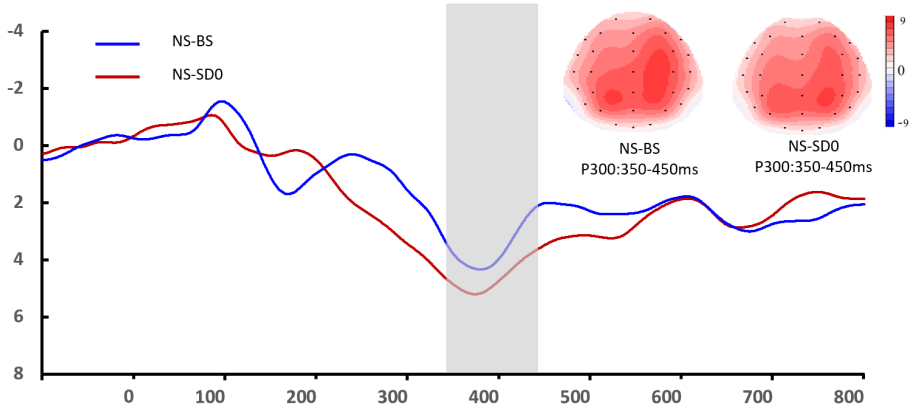


FIGURE 5
Grand mean amplitude of the P3 component in the nocturnal sleep (NS) group at the baseline state (BS) and after 0h of sleep deprivation (SD0). Averaged data from the F3, Fz, F4, C3, Cz, C4, P3, Pz, and P4 electrodes are shown. The topographies correspond to average activity in the time windows (350–450ms, indicated by the gray bar) around the local peaks.

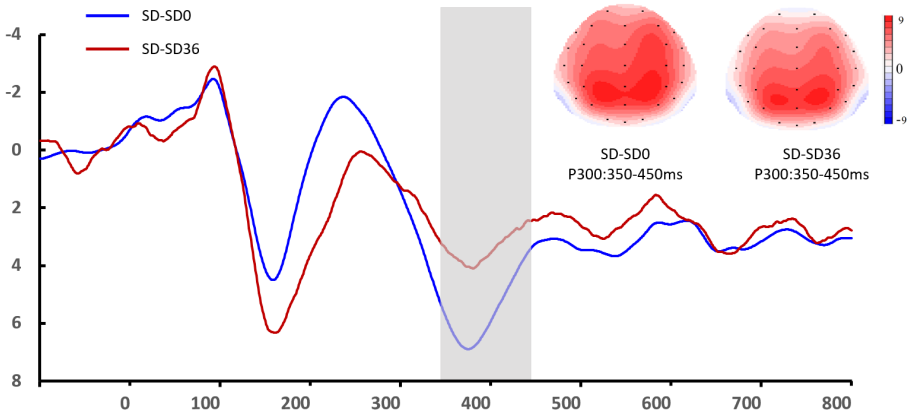


FIGURE 6
Grand mean amplitude of the P3 component in the sleep deprivation (SD) group after 0h of sleep deprivation (SD0) and 36h of sleep deprivation (SD36). Averaged data from the F3, Fz, F4, C3, Cz, C4, P3, Pz, and P4 electrodes are shown. The topographies correspond to average activity in the time windows (350–450ms, indicated by the gray bar) around the local peaks.

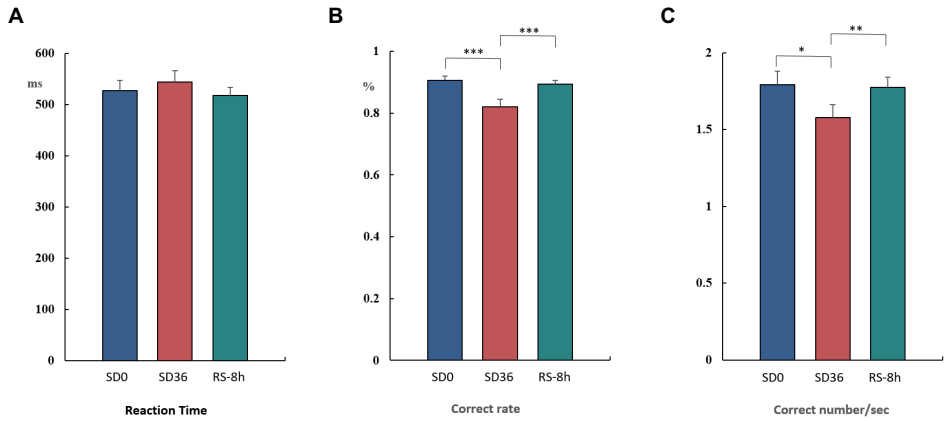


FIGURE 7
Reaction time, rate of correct responses, and number of correct responses per unit time in the three sleep states (mean±standard deviation). SD: sleep deprivation; RS: recovery sleep. *$p$ <0.05, **$p$ <0.01, ***$p$ <0.001.

**TABLE 5** Grand-average peak latency of the N2 and P3 components for correct responses across multiple electrode sites in three sleep states in the sleep deprivation (SD) group.

| | | SD-0h | | SD-36h | | RS-8h | |
|---|---|---|---|---|---|---|---|
| | | N2 | P3 | N2 | P3 | N2 | P3 |
| F3 | M (SD) | 232.57 (36.77) | 372.41 (33.91) | 250.74 (49.32) | 374.85 (33.49) | 243.72 (39.16) | 372.36 (36.55) |
| Fz | M (SD) | 235.89 (38.35) | 370.90 (34.97) | 247.39 (44.22) | 378.39 (32.22) | 242.68 (36.11) | 369.26 (35.48) |
| F4 | M (SD) | 229.21 (36.35) | 371.33 (31.72) | 248.93 (48.36) | 377.63 (33.64) | 238.15 (39.51) | 370.58 (33.27) |
| C3 | M (SD) | 217.58 (33.88) | 367.87 (33.40) | 231.75 (46.67) | 369.35 (29.87) | 225.80 (39.55) | 363.95 (38.51) |
| Cz | M (SD) | 224.36 (36.51) | 361.43 (34.43) | 229.79 (45.19) | 370.04 (34.69) | 230.90 (40.53) | 357.77 (35.88) |
| C4 | M (SD) | 215.79 (35.35) | 368.32 (27.19) | 230.50 (44.68) | 368.90 (34.81) | 229.42 (39.82) | 360.26 (37.63) |
| P3 | M (SD) | 207.55 (45.76) | 359.62 (34.36) | 209.64 (34.93) | 362.05 (40.21) | 211.55 (39.28) | 352.95 (36.65) |
| Pz | M (SD) | 206.46 (31.04) | 353.29 (36.24) | 223.56 (43.61) | 352.26 (37.77) | 211.94 (34.55) | 348.34 (41.02) |
| P4 | M (SD) | 209.79 (46.66) | 334.74 (39.05) | 222.84 (45.27) | 337.95 (45.44) | 212.25 (45.42) | 338.58 (45.31) |

**TABLE 6** Grand-average peak amplitude of the N2 and P3 components for correct responses across multiple electrode sites in three sleep states in the sleep deprivation (SD) group.

| | | SD-0h | | SD-36h | | RS-8h | |
|---|---|---|---|---|---|---|---|
| | | N2 | P3 | N2 | P3 | N2 | P3 |
| F3 | M (SD) | −2.50 (4.17) | 7.80 (3.35) | −3.27 (4.93) | 6.33 (3.21) | −3.35 (2.92) | 7.74 (4.33) |
| Fz | M (SD) | −2.72 (3.95) | 8.31 (3.44) | −3.48 (5.87) | 7.85 (3.87) | −4.03 (3.44) | 8.47 (5.12) |
| F4 | M (SD) | −1.88 (3.66) | 8.93 (3.38) | −2.69 (4.58) | 7.11 (3.85) | −3.06 (3.42) | 9.09 (4.67) |
| C3 | M (SD) | −2.04 (4.03) | 8.49 (3.39) | −2.09 (4.75) | 6.88 (3.56) | −2.30 (2.62) | 8.92 (4.52) |
| Cz | M (SD) | −1.93 (4.51) | 9.64 (3.65) | −1.68 (5.36) | 8.14 (3.28) | −2.20 (3.69) | 10.36 (5.20) |
| C4 | M (SD) | −1.36 (3.63) | 9.05 (3.03) | −0.93 (4.29) | 7.58 (3.38) | −1.08 (3.03) | 10.35 (4.46) |
| P3 | M (SD) | −2.55 (5.08) | 8.51 (3.82) | −2.53 (4.93) | 7.27 (3.55) | −2.81 (4.87) | 8.71 (4.19) |
| Pz | M (SD) | −0.68 (4.17) | 9.20 (4.07) | −0.91 (4.21) | 8.36 (3.68) | −1.05 (4.13) | 9.96 (4.22) |
| P4 | M (SD) | −1.65 (4.03) | 8.00 (3.41) | −0.93 (4.91) | 7.79 (4.41) | −1.60 (3.99) | 8.30 (3.77) |

interaction effect of sleep state and region on the P3 amplitude ($F_{(1.87, 35.55)} = 2.489$, $p = 0.050$, $\eta^2_P = 0.116$). During the three sleep states, the fluctuations in P3 were mainly focused in frontal and central regions (Figure 8). For latency of P3, the main effect of and sleep state was not significant ($F_{(2\ ,\ 38)} = 11.921$, $p = 0.160$, $\eta^2_p = 0.092$). No other main or interaction effect was statistically significant.

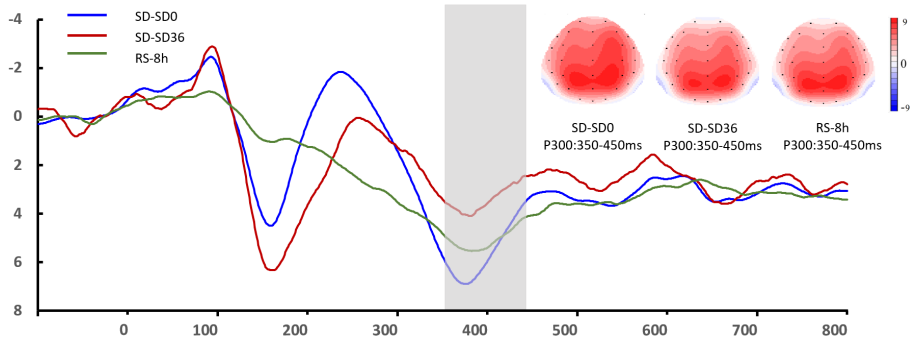


FIGURE 8
Grand mean amplitude of the P3 component in the sleep deprivation (SD) group after 0h of sleep deprivation (SD0), 36h of sleep deprivation (SD36), and 8h of recovery sleep (RS-8h). Averaged data from F3, Fz, F4, C3, Cz, C4, P3, Pz, and P4 electrodes are shown. The topographies correspond to average activity in the time windows (350–450ms, indicated by the gray bar) around the local peaks.

## 4. Discussion

In the present study, we analyzed ERPs to investigate the effects of 8 h of RS on working memory impairment induced by 36 h of TSD. The results of our previous study had revealed that TSD significantly impairs the accuracy of 2-back working memory tasks (Peng et al., 2020). The current behavioral findings demonstrate that 8 h of RS can improve both the accuracy of responses and the number of correct responses per unit time in such tasks. Changes in behavioral indicators effectively reflect the improvement in working memory ability, which may also be related to the recovery of vigilance and disposable attentional resources (Doty et al., 2017). Although a constant cognitive load (2-back) was utilized in the present study, our results indicated that working memory ability improved after 8 h of RS when compared with the performance observed after 36 h of TSD without RS. Moreover, despite there being no marked improvements in reaction time after 8 h of RS, the number of correct responses per unit time increased significantly. Changes in accuracy rates can also influence response times; as such, there may be situations where participants sacrifice accuracy to reduce reaction time (de Bruijn et al., 2020). The number of correct responses per unit of time combines reaction time and accuracy and more accurately reflects an individual's working memory ability and level of cognitive control.

Notably, 8 h of RS after TSD also induced a significant decrease in N2 latency and a significant increase in P3 amplitude. The N2 component is considered to reflect an individual's mental state and level of attention (Schacht et al., 2008), whereas the P3 component is thought to be involved in the decision-making process during cognitive matching tasks (Gosselin et al., 2005). Increases in P3 latency and decreases in P3 amplitude are associated with prolonged wakefulness (Panjwani et al., 2010).In addition, several studies have reported a decrease in reaction time and sustained attention following SD (Rupp et al., 2009; Chua et al., 2014). Thus, our findings are in accordance with previous results and support the notion that 8 h of RS can improve performance and alertness (Zhang et al., 2014). The restoration of attention and alertness following RS may have enabled participants to allocate more attentional resources to working memory tasks, thereby attenuating TSD-induced impairments (Donchin and Fabiani, 1991). Previous studies have also indicated that compared with drowsiness, SD is associated with more pronounced decreases in the activation of the frontoparietal network (which in involved in working memory) (Almklov et al., 2015). Furthermore, SD can reduce metabolic activity in regions associated with information processing and executive control (Choo et al., 2005), whereas RS can restore the overall network organization following TSD (Jiang et al., 2018).

Sustained attention and alertness are essential for the performance of daily activities. Based on the observed changes in the N2 and P3 components (i.e., increased amplitude and decreased latency) after 8 h of RS, we speculated that RS can effectively attenuate impairments in attention and alertness, thus influencing the information integration process. The deterioration of sustained attention seems to be a long-lasting negative effect of SD (de Bruin et al., 2017; Lowe et al., 2017), and is likely caused by decreased arousal of the central nervous system (CNS) (Schneider and Fisk, 1984; Cote et al., 2009). In contrast, more automatic or bottom-up processes appear to be less affected by changes in CNS arousal (Schneider and Fisk, 1984). Therefore, improvements in sustained attention are likely to occur earlier given the greater sensitivity of sustained attention to SD. The completion of a cognitive task usually requires the joint participation of several psychological processes, including early sensory perception, alertness, basic attentional mechanisms, working memory, and decision making. The P3 component appears relatively late, suggesting that it is more reflective of conscious participation and likely involves top-down cognitive control (Kusztor et al., 2019). The observed increases in amplitude and decreases in latency also suggest that 8 h of RS improves the ability to integrate dynamic information during working memory tasks. Communication between the hippocampus and prefrontal areas is vital for the optimal redistribution of temporal memory traces to more resident cortical storage. Therefore, interrupting this communication may impair an individual's ability to form a new memory. In this regard, Chai et al. recently reported that RS re-normalizes hippocampal connections (Chai et al., 2020).

Normal sleep is divided into two phases: rapid eye movement (REM) and slow-wave sleep (SWS). Deep sleep during the N3 stage of SWS is particularly important for restoring mental and physical energy. Following SD, we observed compensatory responses during the restorative sleep stages. Interestingly, the intensity (rather than the duration) of sleep influences the recovery of function following SD. Sleep intensity during SWS is regarded as an indicator of homeostatic sleep pressure (Borbély, 1982; Hennecke et al., 2019). After one night of SD, less than 10 h of RS can sufficiently reduce the level of sleep stress to that observed at the end of a typical 8 h period

of normal sleep (Daan et al., 1984; Achermann and Borbély, 1994). Nevertheless, further increases in the duration of SWS have been observed on the second night of recovery (Carskadon and Dement, 1985). RS exhibits characteristics distinct from those of normal sleep, including a decrease in sleep-onset latency. Key changes have also been observed during the N2 and N3 stages. In addition, longer periods of RS result in a sleep stage distribution similar to that of normal sleep (Hennecke et al., 2019). Therefore, individuals in the RS group experienced increases in the proportion of SWS during RS relative to the amount observed during normal sleep. It is possible that SD-induced impairments in working memory function are specifically attenuated during SWS.

The behavioral and EEG data obtained in this study support our hypothesis that 8 h of RS can attenuate impairments in working memory caused by 36 h of TSD. However, we did not observe significant changes in all the indicators identified in our previous study (Peng et al., 2020). Insufficient sleep may lead individuals to provide conservative estimates of their performance, which may increase the likelihood of compensatory behaviors and protect against the negative consequences of SD (Boardman et al., 2018). Therefore, the results of this study should be interpreted with caution. Previous research has demonstrated that simple cognitive responses are less affected by SD and can be easily recovered following RS, whereas impairments in higher-level cognitive functions are less easily reversed (Nilsson et al., 2005; Skurvydas et al., 2020). Improvements in cognitive function following RS are mainly reflected by changes in alertness and sustained attention, which allow participants to allocate more attentional resources to the current task (Jin et al., 2015). Nonetheless, further studies are required to elucidate the mechanisms by which RS restores cognitive function after TSD. The main goal of this study was to further explore the recovery effect of 8 h of restorative sleep on impaired cognitive ability based on previous findings (i.e., how sleep deprivation impairs working memory or other cognitive functions). In addition, we also included a blank control group, unlike most prior studies.

The present study has some limitations. We did not assess working memory performance using tasks of varying difficulty, which limits our ability to infer how changes in workload impact the restorative effect of RS. In addition, our study included only male volunteers, and caution should be exercised when attempting to extend our findings to female individuals. All participants in this study had good sleep quality; however, the impact of sleep deprivation can differ between the normal population and people with insomnia or rhythm disorders. Rhythm disorders can cause changes in an individual's melatonin secretion cycle, leading to specific deficits in the neurophysiological activity in the attention domain (Gumenyuk et al., 2014). Therefore, our inferences may be limited to an optimally sleeping population. Considering the number of participants in our study and some non-significant findings related to EEG indicators, further studies are required to determine whether 8 h of RS can restore cognitive function to baseline levels. In future studies, we plan to use analytical methods based on the power spectrum. Multimodal studies involving brain network analyses of EEG and imaging data can also help further explain our results. We did not use sleep monitoring technology to investigate whether RS induces specific alterations in sleep structure, necessitating further studies in this regard. Finally, circadian biorhythms are known to affect behavioral performance (Montplaisir, 1981), and their effects should also be considered in future studies.

Our results align with those of previous studies, suggesting that 8 h of RS can partially attenuate the deleterious effects of TSD on working memory. This study provides experimental evidence of the recovery of cognitive function after acute sleep loss. RS has potential value as an applied non-pharmacological strategy to alleviate the effects of sleep deprivation. For example, failure to maintain a high level of alertness may lead to serious consequences during military missions. The widespread use of high-tech equipment demands high levels of cognitive ability and brain function, and sleep deprivation may become more prominent under high-tech warfare conditions in future. Therefore, strengthening research on sleep deprivation and providing effective medical support under continuous combat conditions is of great significance to the success of military endeavors.

## 5. Conclusion

In summary, our results suggest that RS may exert its effects by improving alertness and sustained attention in sleep-deprived individuals. Because SWS dominates the sleep period during RS, these restorative effects are likely to occur during SWS. However, RS had limited effects in the present study, and further studies are required to determine whether 8 h of RS can restore cognitive function to baseline levels.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Ethics statement

The studies involving human participants were reviewed and approved by the Fourth Military Medical University. The patients/participants provided their written informed consent to participate in this study.

## Author contributions

YS, ZP, and YH designed the study. ZP produced the results and wrote the manuscript. LX, HW, SW, and TS contributed data collection and analysis. YY and YS were the guarantors of this study. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



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# Sleep deprivation, sleep fragmentation, and social jet lag increase temperature preference in *Drosophila*

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Despite the fact that sleep deprivation substantially affects the way animals regulate their body temperature, the specific mechanisms behind this phenomenon are not well understood. In both mammals and flies, neural circuits regulating sleep and thermoregulation overlap, suggesting an interdependence that may be relevant for sleep function. To investigate this relationship further, we exposed flies to 12 h of sleep deprivation, or 48 h of sleep fragmentation and evaluated temperature preference in a thermal gradient. Flies exposed to 12 h of sleep deprivation chose warmer temperatures after sleep deprivation. Importantly, sleep fragmentation, which prevents flies from entering deeper stages of sleep, but does not activate sleep homeostatic mechanisms nor induce impairments in short-term memory also resulted in flies choosing warmer temperatures. To identify the underlying neuronal circuits, we used RNAi to knock down the receptor for *Pigment dispersing factor,* a peptide that influences circadian rhythms, temperature preference and sleep. Expressing UAS-*Pdfr*$^{RNAi}$ in subsets of clock neurons prevented sleep fragmentation from increasing temperature preference. Finally, we evaluated temperature preference after flies had undergone a social jet lag protocol which is known to disrupt clock neurons. In this protocol, flies experience a 3 h light phase delay on Friday followed by a 3 h light advance on Sunday evening. Flies exposed to social jet lag exhibited an increase in temperature preference which persisted for several days. Our findings identify specific clock neurons that are modulated by sleep disruption to increase temperature preference. Moreover, our data indicate that temperature preference may be a more sensitive indicator of sleep disruption than learning and memory.

KEYWORDS

**sleep, sleep deprivation, *Drosophila*, temperature preference behavior, sleep fragmentation**

## Introduction

Although the precise function of sleep remains unknown, there is little question that sleep plays an essential role in maintaining the integrity of a large and diverse set of biological systems. In recent years, a main focus of sleep research has been on the relationship between sleep and synaptic plasticity (Stickgold and Walker, 2013; Dissel et al., 2015a; Dissel and Shaw, 2017; Seibt and Frank,

2019; Tononi and Cirelli, 2020; Frank, 2021). Nevertheless, it is important to note that thermoregulation has been implicated in sleep regulation and function from the earliest days of research in the field (De Manaceine, 1894; Kleitman and Doktorsky, 1933; Bentivoglio and Grassi-Zucconi, 1997). Indeed, decades of research have firmly established that sleep and thermoregulation are inextricably intertwined on many levels (Glotzbach and Heller, 1976; Parmeggiani et al., 1983; Szymusiak and McGinty, 1990). Given this intimate interrelationship, it is exciting that recent studies have established that subsets of neural circuits regulating sleep also overlap with circuits regulating thermoregulation (Head et al., 2015; Harding et al., 2018; Szymusiak, 2018; Yadlapalli et al., 2018). However, it is unclear why the same neurons regulate sleep and thermoregulation and whether such overlap is relevant for sleep function.

The interaction between sleep and thermoregulation has clear adaptive value. That is, by coordinating the timing of sleep with daily changes in ambient temperature, animals can avoid extreme conditions and confine waking behaviors to the most optimal times of day (Dillon et al., 2009). Not surprisingly then, circadian mechanisms synergize with sleep and thermoregulatory circuits to regulate behavior. In *Drosophila*, the clock is comprised of 150 neurons that can be divided into two major groups: (1) Lateral neurons (LNd, sLNvs, lLNvs) and (2) Dorsal neurons (DN1, DN2, DN3; Helfrich-Forster, 2003; Taghert and Shafer, 2006; Ma et al., 2021). These neurons have been the topic of intensive investigation and are known to regulate both sleep and temperature regulation (Hamada et al., 2008; Goda and Hamada, 2019; Shafer and Keene, 2021; Schlichting et al., 2022). For example, DN1s are sleep-promoting, coordinate a temperature preference rhythm and can respond to changes in ambient temperature to control the timing of sleep (Kunst et al., 2014; Head et al., 2015; Guo et al., 2016; Tang et al., 2017; Yadlapalli et al., 2018; Alpert et al., 2020). Although clock neurons are known to regulate temperature preference across the biological day, their precise role in mediating the effects of sleep loss remains unclear.

Historically, sleep deprivation has been used as a powerful tool to evaluate sleep regulation and function (Kleitman, 1963; Rechtschaffen et al., 1989a; Bentivoglio and Grassi-Zucconi, 1997). As mentioned, sleep deprivation was used in the earliest of sleep studies where it was found that sleep loss changed temperature regulation. Importantly, sleep deprivation profoundly effects thermoregulation in rodents and humans (Kleitman and Doktorsky, 1933; Rechtschaffen et al., 1989b; Savourey and Bittel, 1994; Shaw et al., 1997). Indeed, feeling cold is an extremely common experience that people report during sleep deprivation (Romeijn et al., 2012). Rats, like humans, also behave as if they feel cold following sleep loss. In fact, rats immediately increase operant responses for heat during sleep deprivation, indicating that changes in thermoregulation are among the earliest detectable changes induced by sleep loss (Shaw et al., 1997). To determine whether the effects of sleep deprivation on temperature regulation are evolutionarily conserved, we evaluated temperature preference in flies following sleep deprivation and sleep fragmentation.

# Methods

## Flies

Flies were cultured at 25°C with 50–60% relative humidity and kept on a diet of yeast, dark corn syrup and agar under a 12-h light:12-h dark cycle. *Tim-GAL4* (BL *#7126*); *Clk.856-GAL4*(BL #93198); *Clk4.1 M-GAL4* (BL #36316)*; Clk4.5F-GAL4* (BL #37526) and *UAS-Pdfr-RNAi* (BL #42508) were obtained from the Bloomington stock center. $per^{01}$, $Pdfr^{503}$, *C929-GAL4;* and *R6-GAL4* were *a* kind gift from Paul Taghert (Washington University in St. Louis).

## Thermal preference

A 30 cm X 2.5 cm aluminum runway was positioned between a hot plate and ice to generate a gradient ranging between 30°C and 17.9°C (Figures 1A,B). Clear Plexiglas walls and ceiling were coated with Fluon (Fisher Scientific #NC0533515) to prevent flies from avoiding contact with the runway by walking on the walls. The chamber was allowed to reach equilibrium for 10 min and the temperature range was verified using an infrared thermometer (Fisher Scientific, 15–077-968; Figure 1C).Thermal preference was assessed in a room at approximately 25°C, a relative humidity of ~50–65% using stable light illumination. Individual flies were then introduced into the chamber for the specified time and their precise temperature was recorded using the infrared thermometer. The temperature range of the thermal gradient was validated between flies. Unless stated otherwise, all experiments were conducted between 2:30–4:30 pm. All experiments were replicated by at least two independent investigators.

## Sleep

Sleep was assessed as previously described (Shaw et al., 2000). Briefly, flies were placed into individual 65 mm tubes containing the same food as they were reared on. All activity was continuously measured through the Trikinetics *Drosophila* Activity Monitoring System ([1]Waltham, MA). Locomotor activity was measured in 1-min bins and sleep was defined as periods of quiescence lasting at least 5 min. All sleep experiments were replicated a minimum of two times.

## Sleep deprivation/restriction

Sleep deprivation was performed as previously described (Shaw et al., 2002; Seugnet et al., 2008). Briefly, flies were placed into individual 65 mm tubes and the sleep-nullifying apparatus (SNAP) was used to sleep deprive or sleep restrict flies Sleep deprivation was performed for 12 h during the dark phase (lights out to lights on). For sleep deprivation, the SNAP was activated once every 20 s for the duration of the experiment. Sleep restriction was performed for 48 h. The SNAP was activated for 18 s once every 15 min for 48 h, yielding a total of 192 stimuli lasting ~60 min; this regime both reduced and fragmented sleep as previously described (Klose and Shaw, 2019). To determine whether the stimulus induced by the SNAP was able to alter temperature preference flies were continually exposed to the SNAP for an equal number of exposures (Bang-Control). Sleep homeostasis was calculated for each individual as a ratio of the minutes of sleep gained above baseline during the 48 h of recovery divided by the total min of sleep lost during 12 h of sleep deprivation.

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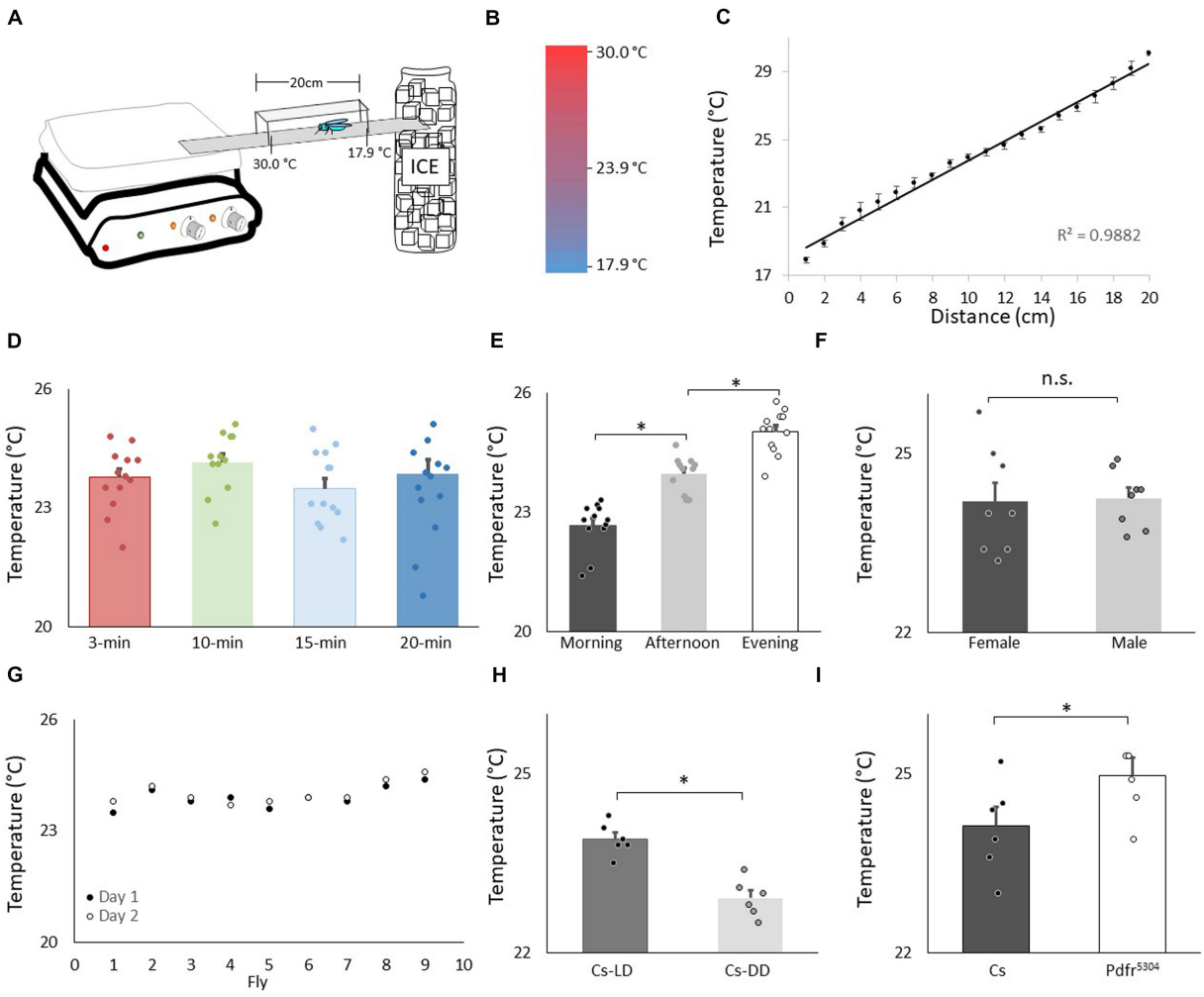


FIGURE 1
Validation of thermal preference assay. **(A)** Schematic for thermal preference apparatus. **(B)** Heat map of the floor surface. **(C)** Temperature readings in 1cm increments measured using an infrared Thermometer (4 independent samples were taken at each location). **(D)** Temperature preference in Canton-S (Cs) flies after being placed into the apparatus for different time intervals ($n$=11 flies/interval); One way ANOVA: $F_{(3,43)}$=1.36; $p$=0.26. **(E)** Temperature preference for Cs flies tested in the morning 8:30–10:30am, $n$=12; afternoon 2-4pm, $n$=11; evening 6-8pm; One way ANOVA for condition: $F_{(2,34)}$=57.4; $p$=2.6$^{E-11}$ *$p$<0.05, corrected Bonferroni test. Power analysis calculates of Cohen's D of 8.28 between morning and afternoon. **(F)** Thermal preference in male and female flies ($n$=8 flies/condition; $p$=0.86, $t$-test). **(G)** Temperature preference in individual flies test on two consecutive days. **(H)** Temperature preference in *Cs* flies tested in flies maintained on a 12:12 Light: dark schedule (LD) and constant darkness (DD) ($n$=6/condition, $p$=9.72$^{E-05}$, $t$-test). Power analysis calculates of Cohen's D of 8.08. **(I)** Temperature preference in *Cs* and *Pdfr*$^{5304}$mutants ($n$=6/condition $p$=0.03, $t$-test).

## Short-term memory

Short-term memory (STM) was assessed by Aversive Phototaxic Suppression (APS) as previously described (Seugnet et al., 2008, 2009). The experimenters were blinded to conditions. In the APS, flies are individually placed in a T-maze and allowed to choose between a lighted and darkened chamber over 16 trials. Flies that do not display phototaxis during the first block of 4 trials are excluded from further analysis (Le Bourg and Buecher, 2002; Seugnet et al., 2009). During 16 trials, flies learn to avoid the lighted chamber that is paired with an aversive stimulus (Filter paper is wetted with $10^{-1M}$ quinine hydrochloride). The performance index is calculated as the percentage of times the fly chooses the dark vial during the last 4 trials of the 16-trial test. In the absence of quinine, where no learning is possible, it is common to observe flies choosing the dark vial once during the last 4 trials in Block 4 (Seugnet et al., 2009). In contrast, flies never choose the dark vial 2 or more times during Block 4 in the absence of quinine (Seugnet et al., 2009). Thus, short term memory is defined as two or more photonegative choices in Block 4. For short term memory experiments following a 12 h sleep deprivation, the deprivation continued until evaluation by the APS. Power analysis using G*Power calculates a Cohen's d of 1.8 and indicates that 8 flies/group are needed to obtain statistical differences (Seugnet et al., 2009).

## Statistics

All comparisons were done using a Student's T-test or, if appropriate, ANOVA and subsequent planned comparisons using modified Bonferroni test unless otherwise stated. Note that a

significant omnibus-F is not a requirement for conducting planned comparisons (Keppel, 1982). All statistically different groups are defined as $^{*}p < 0.05$.

# Results

## Validation of thermal preference

We evaluated temperature preference in adult *Canton-S* (*Cs*) flies using a slightly modified protocol from earlier studies (Sayeed and Benzer, 1996; Hamada et al., 2008). A schematic of the apparatus is shown in Figure 1A. A heat map of the runway floor is shown in Figure 1B. The temperature along the gradient, was measured using an infrared thermometer and is shown in Figure 1C. Importantly, the temperature, was stable, reproducible, and linear, with a slope of 0.6°C/cm, as previously described (Sayeed and Benzer, 1996). Previous studies have evaluated thermal preference in groups of 10–30 flies that are monitored for 20–30 min (Tang et al., 2017; Hague et al., 2020; Ito and Awasaki, 2022). However, we prefer studying individual flies in which (1) the sleep history of that individual has been well characterized, and (2) the individual fly can be retrieved at the end of the assay and their behavior can be further evaluated (Seugnet et al., 2009; Dissel et al., 2015b). Interestingly, when we evaluated individual flies, it appeared as if they settled down and chose a preferred temperature much sooner than 20–30 min. Thus, we examined temperature preference in individual flies in 3 min, 10 min, 15 min and 20 min intervals. As seen in Figure 1D, temperature preference did not change across intervals ranging from 3 to 20 min when tested individually. It is worth noting that the temperature preference of individual flies defined using our protocol closely matches that reported by Sayeed and Benzer (1996).

Evaluating temperature preference in individual flies for 3 min differs from previous studies. Thus, we asked whether we could replicate published findings with our approach. Previous studies indicate that flies display a daily temperature preference rhythm in which they select warmer temperatures across the light period (Kaneko et al., 2012). As seen in Figure 1E, when assessed after 3 min, individual flies select progressively warmer temperatures as the day progresses. Interestingly, previous studies indicate that temperature preference is similar between male and female flies run in groups. As seen in Figure 1F, no sexual dimorphisms were observed in our temperature preference assay when flies were assessed individually. To determine whether the temperature preference of an individual fly was stable, we examined temperature preference in individual flies over two successive day. As seen in Figure 1G, temperature preference is stable in an individual fly across days. A previous report indicates that flies select cooler temperature in the dark compared to siblings maintained in the light (Head et al., 2015). We find similar results using individual flies (Figure 1H). Finally, mutants for the receptor for the *Pigment dispersing factor receptor* ($Pdfr^{5304}$) select warmer temperatures than controls; we find similar results when temperature preference is evaluated for 3 min in individual flies (Figure 1I). Together these data indicate that monitoring thermal preference in individual flies for 3 min accurately identifies thermal preferences and replicates published studies even when using a slightly different protocol.

## Sleep deprivation increase temperature preference

To determine the effects of sleep loss on temperature preference, *Cs* flies were exposed to 12 h of sleep deprivation. As can be seen in Figures 2A,B, sleep deprivation was effective in keeping flies awake and *Cs* flies showed a typical sleep rebound (Shaw et al., 2002). Importantly, sleep deprived flies selected warmer temperatures in the thermal gradient compared to their untreated controls when tested between in the morning (8:30–10:30 am; Figure 2C). Flies also selected warmer temperatures than controls when sleep deprivation was extended into the afternoon and tested for thermal preference between 2:30–4:30 pm (25.4 ± 0.12°C vs. 23.8 ± 0.08°C, respectively, $p < 0.05$). We have previously shown that sleep deprivation disrupts short-term memory and that these memory deficits are completely restored after only 2 h of recovery sleep (Seugnet et al., 2008). Thus, we asked how long it would take for fly's temperature preference to return to baseline. An independent cohort of flies was sleep deprived for 12 h during their primary sleep period. The effectiveness of the sleep deprivation is shown in Figure 2D. Importantly, siblings that were allowed to recover showed sleep rebound (Figure 2E). As seen in Figure 2F, sleep deprivation resulted in flies selecting warmer temperatures replicating the data shown in Figure 2C. Similarly to what we have observed for short-term memory, 2 h of recovery sleep was sufficient to return temperature preference to baseline levels (Figure 2F). Thus, sleep deprivation increases temperature preference in flies as it does in mammals.

## Sleep fragmentation increase temperature preference

We have validated an ethologically relevant sleep fragmentation protocol in flies that can disrupt sleep for extended periods (Klose and Shaw, 2019). The protocol is based upon observations that conditions that support brain plasticity increase daytime sleep-bout duration from ~10 min to ~25 min (Seugnet et al., 2008, 2011; Donlea et al., 2011, 2014; Dissel et al., 2015a). These data suggest that there is a minimum amount of sleep consolidation that is required for sleep to fulfil its functions. Thus, in our sleep fragmentation protocol, flies are kept awake for 1 min every 15 min to limit their ability to obtain restorative sleep. Sleep fragmentation is achieved using the sleep nullifying apparatus as previously described(Klose and Shaw, 2019). As seen in Figures 3A–C, sleep fragmentation modestly disrupts sleep, is effective in preventing long sleep-bouts and does not robustly activate sleep homeostasis. Importantly, sleep fragmentation did not disrupt short-term memory as assessed using Aversive Phototaxic Suppression (APS; Figure 3D). In the APS, flies are individually placed in a T-maze and must learn to avoid a lighted chamber that is paired with an aversive stimulus (quinine/ humidity; Seugnet et al., 2008). The performance index is calculated as the percentage of times the fly chooses the dark vial during the last 4 trials of the 16 trial test and short-term memory is defined as selecting the dark vial on 2 or more occasions during Block 4 (Dissel et al., 2015a,b,c). Despite having a normal short-term memory, flies exposed to 48 h of sleep fragmentation selected warmer temperatures in the thermal gradient compared to untreated siblings when tested in the afternoon (2:30–4:30 pm; Figure 3E). Temperature preference was also increased when flies were exposed to 48 h of sleep fragmentation and tested in the

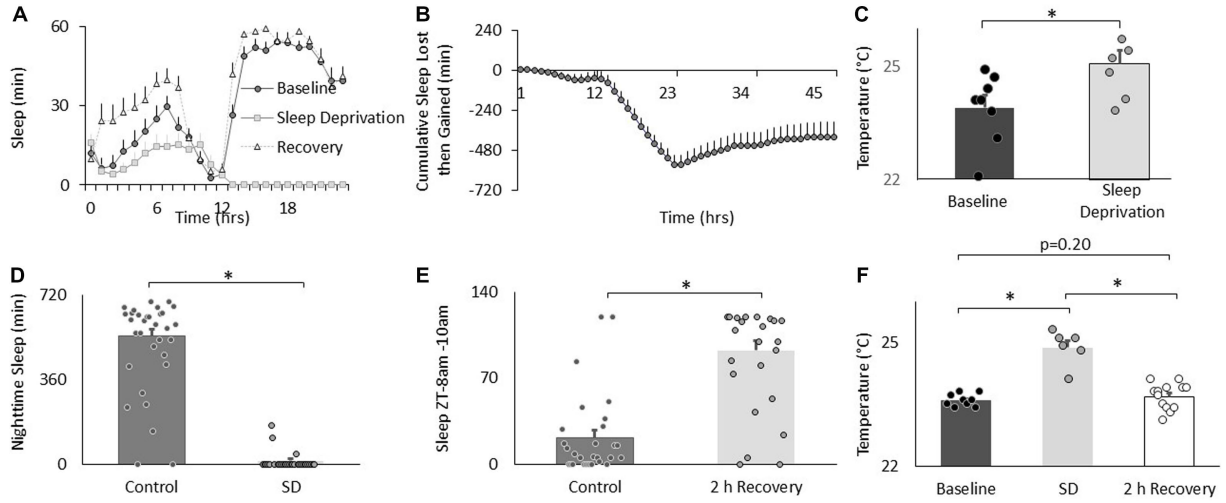


FIGURE 2
Sleep deprivation increases thermal preference. **(A)** Sleep in min/h in *Cs* flies during baseline, sleep deprivation and recovery ($n$=16 flies). **(B)** Cumulative sleep lost then gained plot. **(C)** Temperature preference is increased following 12h of sleep deprivation ($n$=7) compared to baseline ($n$=8) ($p$=0.01, $t$-test). **(D)** Nighttime sleep in untreated controls ($n$=30) compared to sleep deprived siblings ($n$=20; $p=2.40^{E-22}$, $t$-test). **(E)** Sleep in untreated controls ($n$=29) compared to sleep deprived flies during 8-10am 2h sleep recovery period ($n$=20) and ($p=6.88^{E-10}$, $t$-test). **(F)** Thermal preference in baseline ($n$=9), sleep deprivation ($n$=7), and sleep deprivation after 2h of recovery sleep flies (One way ANOVA:$F_{(2,27)}$=39.9; $p=1.62^{E-08}$).

morning (8:30–10:30 am; 25.06 ± 0.06°C vs. 23.4 ± 0.12°C, $p < 0.05$). To exclude the possibility that the stimulus used to keep the animal awake altered temperature preference independently from sleep fragmentation, we exposed flies to the same number of stimuli that accrued during sleep fragmentation but in a consolidated block of 60 min during the light period and immediately evaluated temperature preference. As seen in Figure 3F, no changes in temperature preference were observed. Finally, we examined temperature preference during 2 h, 6 h and 24 h recovery from 48 h of sleep fragmentation. To facilitate comparisons across groups, the timing of recovery was staggered such that temperature preference was evaluated in all groups between 2:30 pm and 4:30 pm. Thus, sleep fragmentation ended at 12:30 pm and 8:30 am for the 2 h and 6 h recovery groups, respectively. The 2:30–4:30 pm time window is ideal since flies can choose either lower or higher temperatures at this time and thereby minimizes the impact of ceiling and floor effects present during the morning and evening time points (Figure 1E; Head et al., 2015). As seen in Figure 3G, temperature preference remained elevated even after 6 h of recovery. Thus, sleep fragmentation increases temperature preference in a thermal gradient and these effects persist for several hours.

## *Pigment dispersing factor receptor* modulates temperature preference following sleep fragmentation

Recent studies highlight the importance of the clock circuitry in regulating light dependent temperature preference (Head et al., 2015). Specifically, light dependent temperature preference was not dependent upon the canonical clock gene *period* (*per*$^{01}$) but was modulated in *Pigment dispersing factor receptor* (*Pdfr*$^{5403}$) mutants. To evaluate whether these genes also play a role in the changes in temperature preference seen after sleep fragmentation, *per*$^{01}$ and *Pdfr*$^{5403}$ mutants were exposed to 48 h of sleep fragmentation and evaluated in the thermal gradient between 2:30 and 4:30 pm as described above. As seen in Figures 4A,B, sleep fragmentation modestly disrupted sleep in *per*$^{01}$ mutants without altering sleep homeostasis. Importantly, *per*$^{01}$ mutants selected warmer temperature in the thermal gradient indicating that the sleep-fragmentation induced changes in temperature preference do not require the molecular clock (Figure 4C). As seen in Figures 4D,E, sleep fragmentation modestly disrupted sleep in *Pdfr*$^{5403}$ mutants without altering sleep homeostasis. However, *Pdfr*$^{5403}$ mutants did not select warmer temperatures in the thermal gradient (Figure 4F), suggesting that *Pdfr*$^{5403}$ may play a role in mediating the effects of sleep fragmentation on temperature preference.

## Clock neurons play a role in temperature preference following sleep fragmentation

The clock is comprised of 150 neurons that can be divided into two major groups. (1) Lateral neurons (LNd, sLNvs, lLNvs) and (2) Dorsal neurons (DN1, DN2, DN3; Helfrich-Forster, 2003; Taghert and Shafer, 2006; Ma et al., 2021). Given the role that clock neurons play in regulating temperature preference (Head et al., 2015; Goda et al., 2016), we evaluated their role in mediating the effects of sleep fragmentation. To begin we used RNAi to knock down *Pdfr* using the pan-clock drivers, *tim-GAL4* and *Clk856-GAL4*. As seen in Figure 5A, sleep fragmentation produced modest changes in sleep time in all genotypes. Importantly, sleep fragmentation increased temperature preference in the thermal gradient for *tim-GAL4/+*, *UAS-Pdfr*$^{RNAi}$/+, *Clk856-GAL4/+* parental controls. However, sleep fragmentation did not alter temperature preference in the thermal gradient in either tim-*GAL4/+ > UAS-Pdfr*$^{RNAi}$/+ or *Clk856-GAL4/+ > UAS-Pdfr*$^{RNAi}$/+ experimental lines (Figure 5B). Together these data indicate that the clock circuitry can influence the impact of sleep fragmentation on temperature preference via the *Pdfr*.

We next evaluated the role of *Pdfr* using GAL4 lines that express in DN1 neurons. DN1 neurons play a role in sleep regulation and modulate

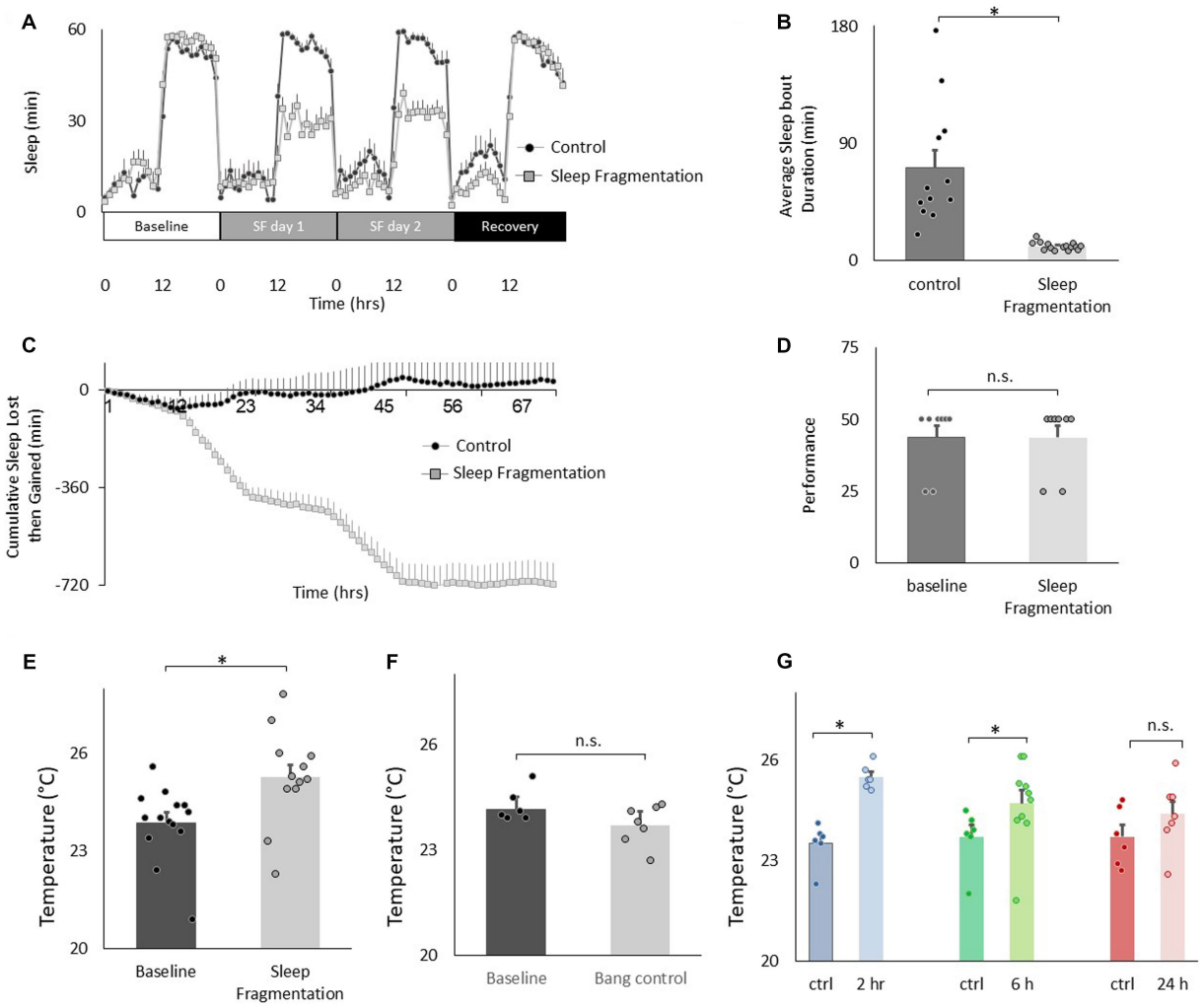


FIGURE 3
Sleep fragmentation increases thermal preference. **(A)** Sleep (min/h) in untreated controls and sleep fragmented siblings ($n$=12 and16 flies/condition). **(B)** Average sleep bout duration during the night in untreated controls and sleep fragmented siblings ($n$=12 and 16 flies/condition, $p$=1.89$^{E-05}$, $t$-test). **(C)** Cumulative sleep lost then gained plot in untreated controls and their sleep fragmented siblings. **(D)** Short-term memory, as assessed using Aversive Phototaxic Suppression, is not impaired by sleep fragmentation ($n$=8 flies/condition, $p$=0.5, $t$-test). **(E)**. Sleep fragmentation increases thermal preference ($n$=12 and 14 flies/condition, $p$=0.004, $t$-test). **(F)** Temperature preference was not changed when flies were exposed to the same number of stimuli as sleep fragmented siblings but did not lose sleep ($n$=6 and 7 flies/condition, $p$=0.065 $t$-test). **(G)** Following sleep fragmentation, flies were allowed to recover for 2h, 6h and 24h. Thermal preference remained elevated for 2h and 6h compared to untreated siblings. One way ANOVA $F_{(3,40)}$=7.3; $p$=0.0005,*$p$<0.05, corrected Bonferroni test.

light-dependent temperature preference (Kunst et al., 2014; Guo et al., 2016; Lamaze et al., 2018; Schlichting et al., 2022). We used RNAi to knock down *Pdfr* using *Clk4.1M-GAL4* and *Clk4.5F-GAL4*. As seen in Figure 6A, sleep fragmentation produced modest changes in sleep time in all genotypes. Interestingly, sleep fragmentation increased temperature preference in the thermal gradient for *Clk4.1M-GAL4*/+, UAS-*Pdfr*$^{RNAi}$/+ and *Clk4.5F-GAL4*/+ parental controls. However, sleep fragmentation did not alter temperature preference in the thermal gradient in either *Clk4.1M-GAL4*/+>UAS-*Pdfr*$^{RNAi}$/+ *or Clk4.5F-GAL4*/+>UAS-*Pdfr*$^{RNAi}$/+ experimental lines (Figure 6B). These data indicate that the DN1s neurons can influence the impact of sleep fragmentation on temperature preference via the *Pdfr*.

Finally, we evaluated the role of the small (sLNvs) and large ventrolateral neurons (lLNvs) in modulating temperature preference after sleep fragmentation. The sLNvs and lLNvs play a role in regulating sleep and waking (Nitabach et al., 2006; Parisky et al., 2008; Sheeba et al., 2008; Chung et al., 2009; Shang et al., 2013). However, the relationship between the sLNvs and lLNvs and temperature preference is more complicated. Initial studies indicated that neither the sLNvs nor the lLNvs influence daytime temperature preference rhythms or light-dependent temperature preference (Kaneko et al., 2012; Head et al., 2015). However, Tang and colleagues report that the sLNvs play a role in setting preferred temperature before dawn (Tang et al., 2017). Interestingly, while pigment dispersing factor (pdf) is expressed in both the sLNvs and lLNvs; the *Pdfr* is only expressed in the sLNvs in unperturbed adult flies (Shafer et al., 2008; Im and Taghert, 2010). However, the *Pdfr* is re-expressed in the lLNvs of adult flies after sleep deprivation, sleep fragmentation and starvation (Klose and Shaw, 2019). Since *Pdfr* is only expressed in the lLNvs of adult flies after a perturbation, its role in regulating temperature preference

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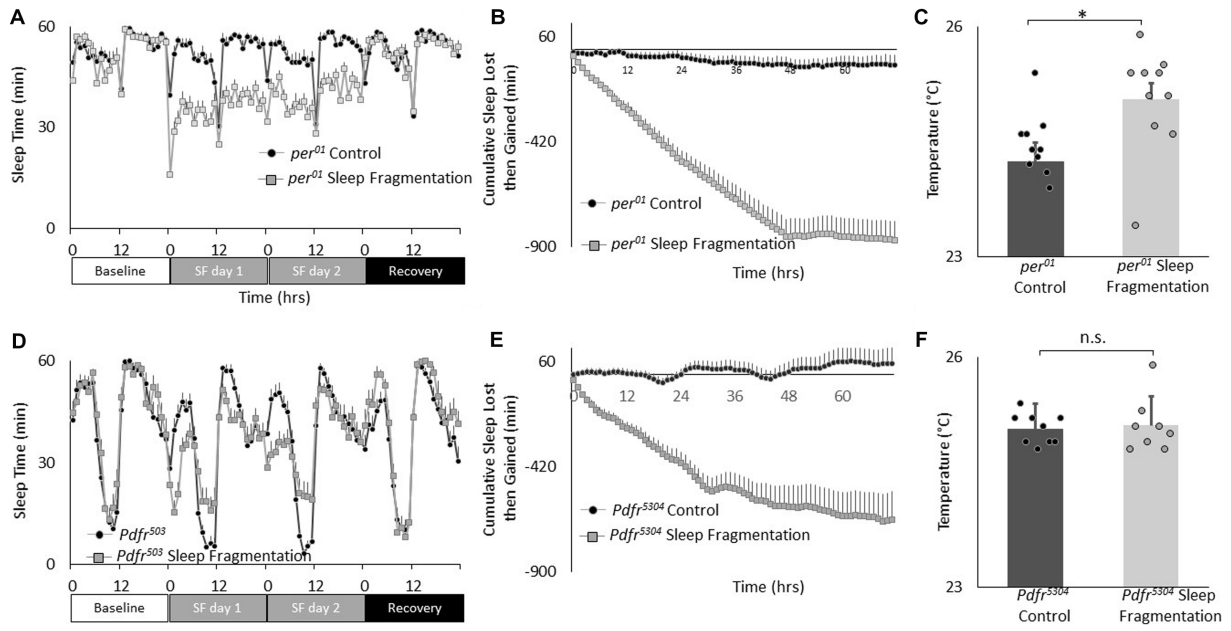


FIGURE 4
Sleep fragmentation alters thermal preference in Pdfr$^{5304}$ mutants. **(A)** Sleep (min/h) in *per*$^{01}$ mutants during baseline, sleep fragmentation and recovery (*n*=16 flies/condition). **(B)** Cumulative sleep lost then gained plot in sleep fragmented *per*$^{01}$ mutants. **(C)** Temperature preference is increased in *per*$^{01}$ following sleep fragmentation (*n*=11 and 10 flies/condition *p*=0. 0.01, *t*-test). **(D)** Sleep in min/h in *Pdfr*$^{5304}$ mutants during baseline, sleep fragmentation and recovery (*n*=16 flies/condition). **(E)** Cumulative sleep lost then gained plot in sleep fragmented *Pdfr*$^{5304}$ mutants. **(F)** Temperature preference is unchanged in *Pdfr*$^{5304}$ following sleep fragmentation (*n*=7 flies/condition *p*=0.47, *t*-test).

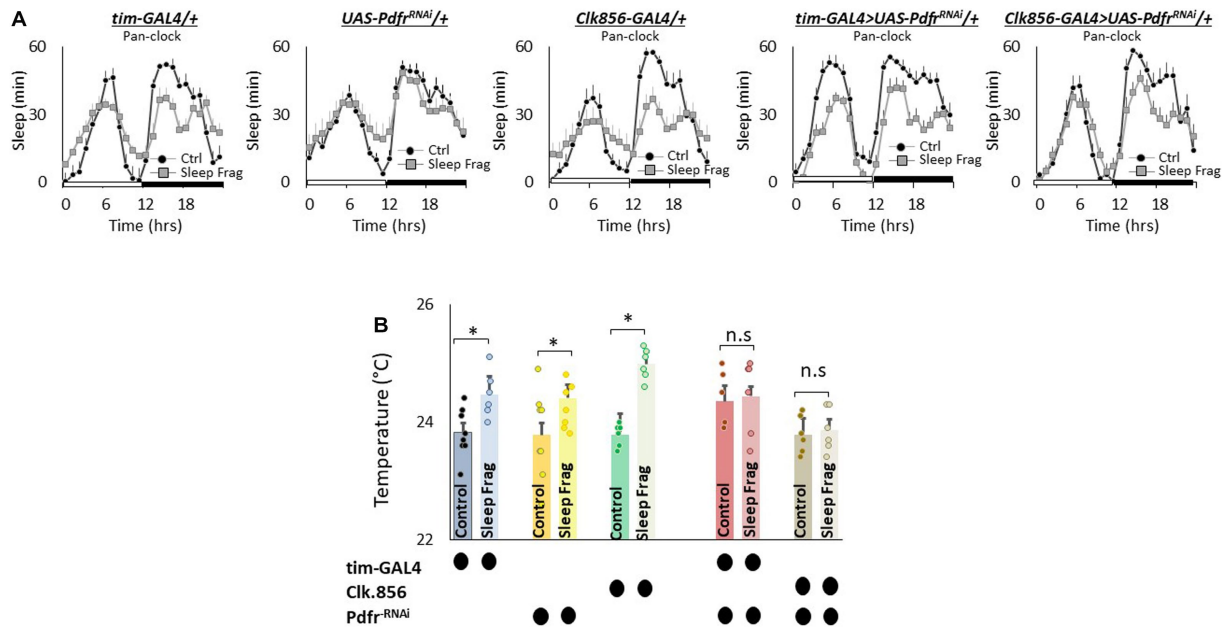


FIGURE 5
Knocking Down *Pdfr* in Clock Neurons Prevents Changes in Thermal Preference Induced by Sleep Fragmentation. **(A)** Sleep in min/h in *tim-GAL4/+*, *UAS-Pdfr*$^{RNAi}$*/+*, *Clk856-GAL4/+*, *tim-GAL4/+>UAS-Pdfr*$^{RNAi}$*/+and Clk856-GAL4/+>UAS-Pdfr*$^{RNAi}$*/+*during baseline (Ctrl) and sleep fragmentation (*n*=16 flies/condition). **(B)** Sleep fragmentation increases temperature preference *tim-GAL4/+*, *UAS-Pdfr*$^{RNAi}$*/+*, *Clk856-GAL4/+* parental controls. Temperature preference in *tim-GAL4/+>UAS-Pdfr*$^{RNAi}$*/+and Clk856-GAL4/+>UAS-Pdfr*$^{RNAi}$*/+* is not altered by sleep fragmentation; A 5(genotype) X 2(condition) ANOVA $F_{(4,57)}$=2.92; *p*=0.029,**p*<0.05, corrected Bonferroni test. Temperature preference in untreated experimental lines (Ctrl) do not differ from parental controls, One-way ANOVA for genotype $F_{(4,34)}$=1.68; *p*=0.17; corrected Bonferroni test for *tim-GAL4/+>UAS-Pdfr*$^{RNAi}$*/+* *p*=0.17 and *p*=0.24 *and Clk856-GAL4/+>UAS-Pdfr*$^{RNAi}$*/+* *p*=1 and *p*=0.80.

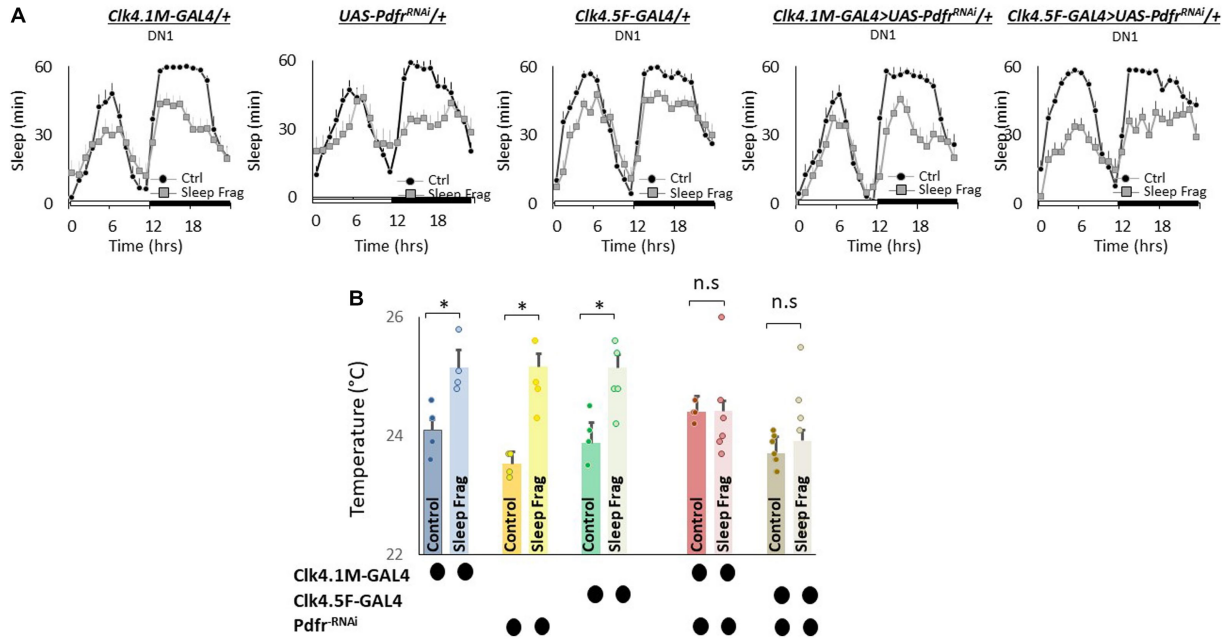


FIGURE 6
Knocking Down *Pdfr* in dorsal Clock Neurons (DN1s) neurons Prevents Changes in Thermal Preference induced by Sleep Fragmentation. **(A)** Sleep in min/h in *Clk4.1M-GAL4/+*, *UAS-Pdfr$^{RNAi}$/+*, *Clk4.F5-GAL4/+*, *Clk4.1M/+>UAS-Pdfr$^{RNAi}$/+and Clk4.5F-GAL4/+>UAS-Pdfr$^{RNAi}$/+*during baseline (Ctrl) and sleep fragmentation ($n$=16 flies/condition). **(B)** Sleep fragmentation increases temperature preference in *Clk4.1-GAL4/+*, *UAS-Pdfr$^{RNAi}$/+*, *Clk4.F5-GAL4/+* parental controls. Temperature preference in *Clk4.1-GAL4/+>UAS-Pdfr$^{RNAi}$/+and Clk4.F5-GAL4/+>UAS-Pdfr$^{RNAi}$/+* is not altered by sleep fragmentation; A 5(genotype) X 2(condition) ANOVA $F_{(4,45)}$=3.02; $p$=0.021,*$p$<0.05, corrected Bonferroni test. Temperature preference in untreated experimental lines (Ctrl) do not differ from both parental controls, One-way ANOVA for genotype $F_{(4,27)}$=5.2; $p$=0.003; corrected Bonferroni test for *Clk4.1-GAL4/+>UAS-Pdfr$^{RNAi}$/+*; $p$=0.24 and $p$=0.24 and *Clk4.F5-GAL4/+>UAS-Pdfr$^{RNAi}$/+*; $p$=0.24 and $p$=0.08.

remains unexplored. Thus, we used *UAS-Pdfr$^{RNAi}$* to knock down the *Pdfr* in sLNvs (*R6-GAL4*) and lLNvs (*c929-GAL4*). As seen in Figure 7A, sleep fragmentation produced modest changes in sleep time in all genotypes. Interestingly, sleep fragmentation increased temperature preference in the thermal gradient for *R6-GAL4/+*, UAS-*Pdfr$^{RNAi}$*/+ and *c929-GAL4/+* parental controls (Figure 7B). However, sleep fragmentation did not alter temperature preference in the thermal gradient in either *R6-GAL4/+* > UAS-*Pdfr$^{RNAi}$*/+ *or c929-GAL4/+* > UAS-*Pdfr$^{RNAi}$*/+ experimental lines. These data indicate that both the sLNvs and the lLNvs can influence the impact of sleep fragmentation on temperature preference via the *Pdfr*.

## Social jet lag increases temperature preference

Social jet lag is a misalignment between an individual's internal biological clock and their social schedule. Social jet lag can occur when people wake up early for work during the week and then stay up later and sleep in on the weekends to make up for lost sleep (Juda et al., 2013; Pilz et al., 2018; Reis et al., 2020; Fischer et al., 2021). Social Jet lag can be modeled in *Drosophila* where it has been shown to dampen rhythmicity in most circadian neurons (Nave et al., 2020). Thus, we explored the effects of social jet lag on temperature preference in *Cs* flies. To induce social jet lag, the timing of lights-off is delayed by 3 h on Friday night and then shifted back 3 h on Sunday night to mimic a weekend schedule (Figure 8A, arrows). As can be seen in Figure 8A, sleep in flies exposed to Social Jet Lag closely resembles that seen in their age-matched untreated controls beginning on Monday after the light schedule has returned to normal to mimic a typical work week schedule. Indeed, quantification of sleep parameters, including total sleep time and sleep consolidation (average sleep bout duration during the day and average sleep bout duration at night) do not differ between flies exposed to social jet lag and their controls after being placed on a work-week schedule (Figure 8B). To further explore how social jet lag alters sleep, we evaluated metrics designed to measure sleep pressure $P_{Doze}$ and sleep depth $P_{Wake}$ (Wiggin et al., 2020). As seen in Figure 8C, social jet lag did not alter either metric compared to untreated siblings. Social Jet lag has been reported to disrupt short-term memory (Nave et al., 2020). We replicate that finding here (Figure 8D). In addition, to memory impairment, we show that flies exposed to social jet lag choose warmer temperatures in a thermal gradient when tested in the afternoon (2:30–4:30 pm; Figure 8D). Flies exposed to social jet lag also select warmer temperatures when tested in the morning (8:30–10:30 am; 23.4 ± 0.24°C vs. 24.2 ± 0.08°C, $p$ < 0.05). Together, these data indicate that social jet lag disrupts both learning and memory and temperature preference.

To determine how long the effects of social jet lag might persist, we evaluated sleep, short-term memory and temperature preference on Friday. Recall that the light schedule had been returned to baseline on Sunday evening. As seen in Figures 8E,F measures of sleep time, sleep architecture and sleep depth are not altered by social jet lag compared to untreated controls. Despite normal sleep metrics, social jet lag treated flies shown memory impairments and increases in temperature preference several days after being placed on their typical

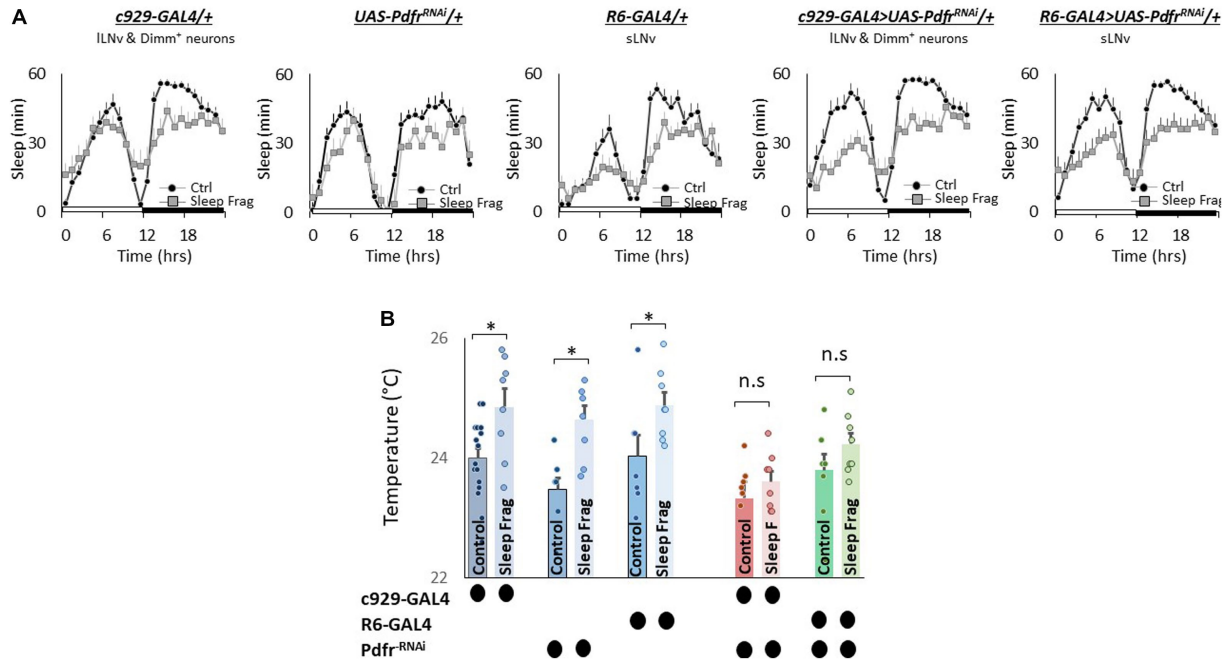


FIGURE 7
Knocking Down *Pdfr* in ventral Lateral (LNvs) neurons Prevents Changes in Thermal Preference Induced by Sleep Fragmentation. **(A)** Sleep in min/h in *c929-GAL4/+*, *UAS-Pdfr$^{RNAi}$/+*, *R6-GAL4/+*, *c929/+>UAS-Pdfr$^{RNAi}$/+and R6-GAL4/+>UAS-Pdfr$^{RNAi}$/+*during baseline (Ctrl) and sleep fragmentation ($n$=16 flies/condition). **(B)** Sleep fragmentation increasses temperature preference *c929-GAL4/+*, *UAS-Pdfr$^{RNAi}$/+*, *R6-GAL4/+* parental controls. Temperature preference in *c929-GAL4/+>UAS-Pdfr$^{RNAi}$/+* and *R6-GAL4/+>UAS-Pdfr$^{RNAi}$/+* is not altered by sleep fragmentation; A 5(genotype) X 2(condition)ANOVA $F_{(4,77)}$=1.12; $p$=0.35,*$p$<0.05, corrected Bonferroni test. Temperature preference in untreated experimental lines (Ctrl) do not differ from both parental controls, One-way ANOVA for genotype $F_{(4,46)}$=1.67; $p$=0.17; corrected Bonferroni test for *c929-GAL4/+>UAS-Pdfr$^{RNAi}$/+*; $p$=0.03 and $p$=0.68 and *R6-GAL4/+>UAS-Pdfr$^{RNAi}$/+*; $p$=0.36 and $p$=0.59.

weekday schedule (Figure 8G). These data indicate that social jet lag results in long-lasting changes to short-term memory as well as temperature preference.

## Discussion

Our data indicate that the effects of sleep deprivation on thermoregulation are evolutionarily conserved. In addition, we show that even small disruptions in sleep induced by sleep fragmentation are able to alter thermoregulatory centers. Furthermore, we localize the effects of sleep disruption to subsets of clock neurons that play dual roles in sleep and thermoregulation. Finally, we show that while clock circuits play a role in mediating the effects of sleep disruption on temperature preference, changes in temperature preference do not require a functioning molecular clock.

The success of sleep deprivation as a tool to study sleep function depends upon the ability to monitor relevant outcome variables and their underlying neuronal circuitry. However, sleep deprivation alters a variety of physiological processes including learning and memory, metabolic, immune, digestive, cardiovascular, respiratory, and endocrine systems, to name a few (Rechtschaffen et al., 1989b; Spiegel et al., 1999; Imeri and Opp, 2009). Each of these systems are under complex regulatory control and require specialized equipment and training to evaluate properly. In contrast, temperature preference is an evolutionarily relevant outcome-variable that can be quantified using equipment found in most labs. Importantly, the temperature preference assay is exceedingly robust and the effect sizes that are seen following sleep disruption are notably high. For example, calculating the effects size for observing a difference between control and sleep deprived siblings using short-term memory reveals a Cohen's D of 1.8, a result seen for other performance metrics in humans (Frey et al., 2004; Seugnet et al., 2009). However, when examining the impact of sleep deprivation or sleep restriction, on temperature preference the Cohen's D is substantially higher (Cohen's D > 3). Thus temperature preference is a unique outcome variable that can be evaluated following sleep disruption to address sleep function.

Historically, temperature preference is conducted on groups of 20–30 flies tested together as a group for 30 min. To our surprise, individual flies quickly settled down in the thermal gradient after ~3 min. The temperatures individual flies chose are remarkably similar to the values obtained when flies were evaluated in groups (Sayeed and Benzer, 1996; Hamada et al., 2008). Importantly, we were able to replicate key findings from previously published manuscripts. Furthermore the temperature chosen by a fly was stable across days. The data presented here were collected by two independent investigators indicating that this assay produces consistent, reliable outcomes that are easily reproduced. Together, our results indicate that temperature preference in flies is a robust phenotype that can return similar results even when the protocol is changed modestly between labs.

In contrast to sleep deprivation, sleep fragmentation disrupts sleep consolidation without inducing a strong homeostatic response or deficits in short-term memory (Klose and Shaw, 2019). Nonetheless, sleep fragmentation is not without its consequences. Indeed, we have previously shown that sleep fragmentation can alter the expression of

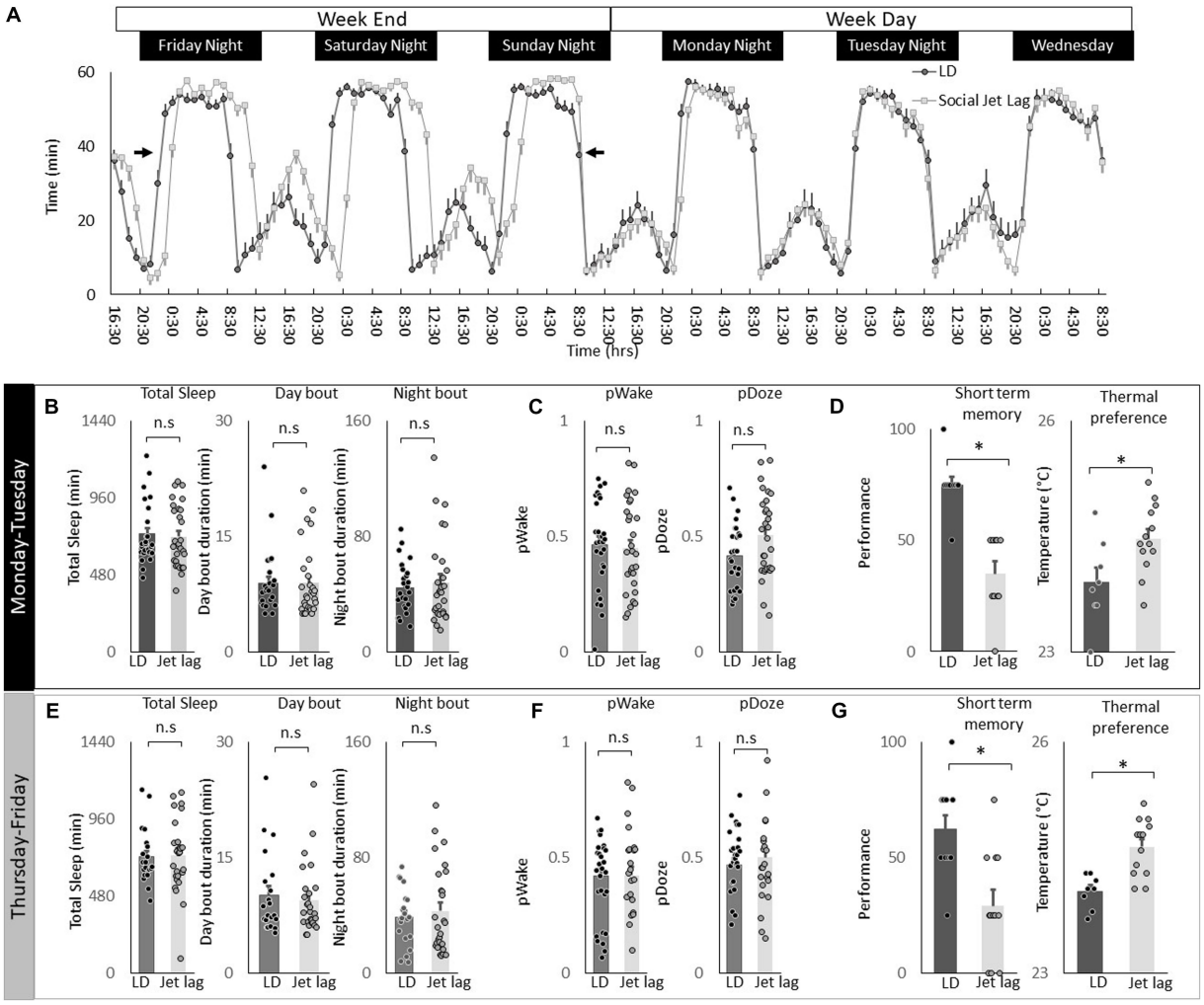


FIGURE 8
Social Jet Lag increases Thermal Preference. **(A)** Sleep in min/h in *Cs* flies maintained on a 12:12 LD schedule and siblings that have been exposed to social jet lag. For social jet lag, the timing of lights out was delayed 3h on Friday night and then advanced back 3h on Sunday night (arrows). **(B)** Social Jet lag did not disrupt total sleep time, or sleep bout duration measured during the day or night compared to untreated siblings ($n$=28 and 30 flies/condition, $p$>0.05, $t$-test). Data are presented from Monday morning at lights-on to Tuesday morning at lights-on. **(C)** Social Jet lag did not disrupt $P_{Wake}$ or $P_{Doze}$ ($n$=28,30 flies/condition, $p$>0.05, $t$-test). **(D)** Social jet lag impaired short-term memory compared to untreated siblings ($n$=10 flies/condition) *$p$=5.63$^{E-06}$. Flies exposed to social jet lag selected warmer temperatures in the thermal gradient compared to controls ($n$=8 and13 flies/condition, *$p$=0.01, $t$-test). **(E)** Social Jet lag did not disrupt total sleep time, or sleep bout duration measured during the day or night compared to untreated siblings ($n$=28 and 30 flies/condition, $p$>0.05, $t$-test). Data are presented from Thursday morning at lights-on to Friday morning at lights-on. **(F)** Social Jet lag did not disrupt $P_{Wake}$ or $P_{Doze}$ ($n$=28 and 30 flies/condition, $p$>0.05, $t$-test). **(G)** Social jet lag impaired short-term memory compared to untreated siblings ($n$=12 flies/condition; *$p$=0.001, $t$-test). Flies exposed to social jet lag selected warmer temperatures in the thermal gradient compared to controls ($n$=13 and 16 flies. Condition, *$p$=0.0007, $t$-test).

the *Pdfr* within the clock circuitry of adult flies. In the current study, we show that sleep fragmentation does not alter temperature preference in $Pdfr^{5403}$ mutants or when *Pdfr* is knocked down in clock neurons. Within the clock circuitry, subsets of neurons regulate different aspects of temperature preference (Goda and Hamada, 2019). For example, light dependent temperature preference is mediated by DN1s, while body temperature rhythms are modulated by DN2s in concert with the sLNvs (Head et al., 2015; Goda et al., 2016). Surprisingly, expressing PDFR in DN1s restores light dependent temperature preference in $Pdfr^{5304}$ mutants even though mutants for the neuropeptide, Pigment dispersing factor ($pdf^{01}$), display normal temperature preference (Head et al., 2015). Flies mutant for *Diuretic hormone 31*, a putative ligand for the PDFR, also display normal light dependent temperature preference leaving the mechanisms leading to the activation of the PDFR unknown in this context (Head et al., 2015). Given these observations, it is possible that the changes in temperature preference we observe following sleep fragmentation may not be due exclusively to PDF. However, sleep fragmentation impacts additional sets of clock neurons compared to those utilized during light dependent temperature preference. The clock neurons modulated by sleep fragmentation include the lLNvs that only express the PDFR following challenges that increase sleep drive (Klose and Shaw, 2019) as well as the sLNvs. Nonetheless, the finding that $pdf^{01}$ mutants maintain their ability to modulate light dependent temperature preference indicates that light, and perhaps sleep fragmentation, can alter clock circuitry, and thus temperature preference via recruiting

additional neurotransmitters (e.g., glutamate, dopamine) or peptides (e.g., sNPF). Consistent with this hypothesis, ambient temperature feeds back on specific clock neurons to sculpt the timing of sleep and waking across the biological day (Yadlapalli et al., 2018; Alpert et al., 2020; Fan et al., 2022). Together, these observations highlight the interconnectedness of clock neurons and suggest that sleep fragmentation may disrupt temperature preference by altering the balance of activity within the clock circuit via a variety of peptides/transmitters (Chen et al., 2022; Reinhard et al., 2022; Shafer et al., 2022; Sun et al., 2022).

In humans, social jet lag disrupts circadian rhythms and results in a variety of adverse health outcomes (Foster et al., 2013; Chellappa et al., 2019; Reis et al., 2020). The social jet lag protocol we used was designed to mimic sleep/wake schedules commonly seen in humans (Nave et al., 2020). This protocol dampens rhythmicity in most clock neurons and alters sleep regulation (Nave et al., 2020). We show that social jet lag disrupts short-term memory and increases temperature preference. In addition, we show that the impact of social jet lag are long lasting. That is, while the light schedule is restored on Sunday evening, flies display alterations to short-term memory and temperature preference that persist at least until the following Friday. If flies were maintained on this schedule through the subsequent weekend, as is the case for many humans, they might not fully recover.

We chose to focus on *Pdfr* as a mediator of sleep loss induced changes to temperature preference given its ability to impact such a diverse set of neurons. Ongoing studies are underway to determine whether the effects of social jet lag are mediated by *Pdfr* and to identify the underlying circuitry. As noted above, PDF can modulate the timing of behavior by staggering when, during the biological day, neurons display peak activity (Liang et al. 2017). PDF modulates the timing of peak activity both within subsets of neurons in the clock circuit and on their downstream output targets (Liang et al., 2016, 2023). Thus social jet lag has the opportunity to disrupt short-term memory and temperature preference by disrupting a large set of diverse neurons in flies. Social jet lag has a seemingly large reach in humans as well, adversely effecting cardiovascular disease, metabolic disorders and mood. Identifying the mechanisms used by social jet lag to disrupt temperature preference in flies may provide new clues into how social jet lag adversely impacts a variety of physiological systems that are relevant for human health.

It is important to note that mechanisms regulating sleep and waking are plastic such that sleep and wake promoting neurons modulate their response properties to match sleep need with environmental demands (Rattenborg et al., 2004; Lyamin et al., 2005; Keene et al., 2010; Thimgan et al., 2010; Lesku et al., 2012; Gravett et al., 2017; Machado et al., 2017). For example, the wake-promoting lLNvs do not typically express the *Pdfr* in healthy adults (Shafer et al., 2008). However, the *Pdfr* is re-expressed in the lLNvs during conditions of high sleep drive, and the presence of the *Pdfr* in the lLNvs plays an important for maintaining adaptive behavior (Klose and Shaw, 2019). Similarly, the *Dopamine 1-like receptor 1* (*Dop1R1*) is not expressed in the sleep-promoting dorsal Fan Shaped Body neurons in healthy adults (Pimentel et al., 2016). However, following starvation or time restricted feeding the *Dop1R1* is recruited to the dorsal Fan Shaped body to provide additional inhibitory tone to sleep promoting neurons (Dissel et al., 2022). Finally, a sleep circuit that is primarily active during a narrow developmental time-window can be reactivated in adults when flight is impaired (Melnattur et al., 2020). These findings imply that it might be important to re-examine signaling pathways that have been previously ruled out as relevant to sleep regulation in order to better understand the effects of sleep disturbance on temperature preference. Nonetheless, understanding sleep plasticity will be important for fully understanding sleep regulation and function, and may provide crucial insight into elucidating the molecular mechanisms induced by sleep deprivation and sleep fragmentation.

The function of sleep has puzzled scientists for decades. We propose that the evolutionary origins of sleep may be better understood by determining how sleep deprivation affects neurons controlling thermoregulation in the poikilothermic fly. Both euthermic and poikilothermic animals use behavioral thermoregulation to accomplish similar goals. For example, both poikilothermic and euthermic animals can use behavior to adjust body temperature and thus conserve energy. Similarly, both poikilothermic and euthermic animals can use behavioral thermoregulation to optimize their ability to avoid predators, engage in reproductive behavior, or select the best environment for sleep (Goda and Hamada, 2019; Harding et al., 2019). Indeed, ambient temperature has a profound effect on sleep in natural environments (Yetish et al., 2015; Gravett et al., 2017). Moreover, animals are known to engage in a number of behavioral adaptations that allow them to sleep in suboptimal thermal environments (Rakotomalala et al., 2017; Campbell et al., 2018; Harding et al., 2019; Reinhardt et al., 2019; Mills et al., 2021). Thus, the interaction between sleep and behavioral thermoregulation is evolutionarily conserved. We expect that understanding the molecular mechanism linking sleep with temperature regulation in flies will provide insight into sleep regulation and function in mammals.

## Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

## Author contributions

SR, MF, VS, VL, HF, ZL EP, DA, IH, AK, and PS performed experiments. SR, MF, and PS oversaw experiments, analysis, and project direction. PS designed the experiments. SR, MF, EP, and PS wrote the paper. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2023.1175478/full#supplementary-material

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# Moderate-to-high risk of obstructive sleep apnea with excessive daytime sleepiness is associated with postoperative neurocognitive disorders: a prospective one-year follow-up cohort study

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**Background:** Few studies found that obstructive sleep apnea (OSA) may be related to postoperative neurocognitive disorders (PND) including postoperative delirium (POD) and cognitive decline (POCD) in the early postoperative period. However, the results are controversial and need further verification, and no research has explored the effect of OSA on the incidence of PND during the 1-year follow-up periods. Furthermore, OSA patients with excessive daytime sleepiness (EDS) as a severe phenotype have more significant neurocognitive impairments, but the relationship between OSA with EDS and PND within 1 year after surgery has not been studied.

**Objectives:** To explore the effect of moderate-to-high risk of OSA and the moderate-to-high risk of OSA with EDS on PND within 1 year after surgery.

**Methods:** In this prospective cohort study, including 227 older patients, moderate-to-high risk of OSA (using STOP-BANG), subjective EDS (using Epworth Sleepiness Scale), and objective EDS (using Actigraphy) were selected as exposures. Key outcomes included POD during hospitalization (using Confusion Assessment Method-Severity), POCD at discharge, 1-month and 1-year after surgery (using Mini-Mental State Examination and Telephone Interview for Cognitive Status-40). We applied multiple logistic regression models to estimate the effect of moderate-to-high risk of OSA and moderate-to-high risk of OSA with EDS on PND.

**Results:** In the multivariate analysis, moderate-to-high risk of OSA was not associated with POD during hospitalization and POCD at discharge, 1-month, and 1-year after surgery ($p>0.05$). However, the moderate-to-high risk of OSA with subjective EDS was related to POCD at discharge compared to the moderate-to-high risk of OSA or normal group (no moderate-to-high risk of OSA and no EDS) ($p<0.05$). In addition, moderate-to-high risk of OSA with objective EDS was associated with POCD at discharge, 1-month, and 1-year postoperatively compared to the moderate-to-high risk of OSA or normal group ($p<0.05$).

**Conclusion:** Moderate-to-high risk of OSA with EDS, not moderate-to-high risk of OSA alone, was a clinically helpful predictor for POCD within 1-year after surgery and should be routinely assessed before surgery.

KEYWORDS

**obstructive sleep apnea, excessive daytime sleepiness, perioperative neurocognitive disorders, older adults, surgery**

## Introduction

Obstructive sleep apnea (OSA), characterized by chronic intermittent hypoxemia and sleep fragmentation, is primarily caused by episodes of complete or partial airway obstruction during sleep (Malhotra and White, 2002) OSA occurs in 10%–20% of the population, increases with age, and peaks after 60 (Bixler et al., 1998, 2001; Peppard et al., 2013). Excessive daytime sleepiness (EDS) is the most common symptom in patients with OSA, and its clinical features are daytime drowsiness, reduced wakefulness, and decreased vigilance (Sauter et al., 2000). EDS has a prevalence of 16%–22% in OSA patients and often means patients at high risk of OSA. Previous studies showed that OSA patients with EDS as a distinct clinical phenotype had lower oxygen saturation and more sleep disturbance than those without EDS (Zhou et al., 2016). Many studies recommended Epworth Sleepiness Scale (ESS) for measuring subjective EDS and multiple sleep latency test (MSLT) for objective EDS, respectively (Ren et al., 2016). Daytime naps recorded by actigraphy also reflect objective EDS, (Leng et al., 2018) which is a compensatory reaction to nocturnal sleep fragmentation, and it can also be a marker of EDS in older adults (Hsieh et al., 2016).

Previous studies have shown that OSA is associated with impaired cognitive function, including attention, alertness, memory, and executive function (Andrade et al., 2018; Stepnowsky et al., 2019; Bubu et al., 2020; Léger and Stepnowsky, 2020; Liu et al., 2020). EDS may also contribute to cognitive impairment in OSA patients, especially attention and memory function because EDS is strictly related to sleep deprivation and fragmentation (Steiropoulos et al., 2019). Many studies reported that OSA patients with EDS have more significant cognitive impairments involving attention and vigilance, learning and memory, and executive function than those without EDS (Zhou et al., 2016). Several pathophysiological factors, such as intermittent hypoxia, systemic inflammation, and oxidative stress, may influence cognitive function in OSA patients. It has been speculated that the interaction between EDS and hypoxemia contributes to neurocognitive deficits (Roche, 2016).

The prevalence of OSA in the surgical population is higher than in the normal population and varies widely among different surgery patients (Van Onselen et al., 2013; Zaremba et al., 2016). Multiple perioperative factors such as anesthetics and analgesics can worsen OSA. In addition, reorganization of sleep architecture with rapid eye movement sleep rebound attributed to postoperative disrupted, reduced, and poor-quality sleep leads to exacerbation of OSA and EDS (Finkel et al., 2009; Young et al., 2009). Add to them downstream reactions of surgery stress and anesthetics such as inflammatory responses, oxidative stress, metabolic disorders, and neuronal damage, the postoperative complications are more common in surgical populations with OSA than those without OSA (Memtsoudis et al., 2011; Roggenbach et al., 2014; Lam et al., 2017; Chan et al., 2019).

In recent years, OSA as a substantial risk factor for postoperative delirium and cognitive decline has aroused scholars' attention. Postoperative neurocognitive disorder (PND) is a term that refers to cognitive impairment associated with anesthesia and surgery, including the acute event (postoperative delirium, POD) and cognitive decline diagnosed up to 1 year after surgery (postoperative cognitive dysfunction, POCD) (Evered et al., 2018). POD occurs in 13%–34% of surgical patients, and POCD occurs in 7%–50%, depending on surgery type and surgical populations (Postler et al., 2011; Norkienė et al., 2013; Krenk et al., 2014; Aceto et al., 2015; Goettel et al., 2017; Kotfis et al., 2018; Wang C. G. et al., 2018; Chaiwat et al., 2019; Guo et al., 2020; Kang et al., 2020; Wang et al., 2020; Yang et al., 2020; Li et al., 2021). Previous studies showed that the prevalence of POD and POCD were 14% and 19% among elderly patients undergoing gastrointestinal surgery, respectively (Yang et al., 2020; Li et al., 2021). Enough evidence has shown that the development of PND is associated with inflammation, oxidative stress, metabolic disorders, increased blood–brain barrier permeability, and neuronal damage (Hudetz et al., 2011; Subramaniyan and Terrando, 2019; Lin et al., 2020). Patients who developed PND have an increased risk of postoperative complications and a worse prognosis (Abelha et al., 2013; Abawi et al., 2016; Lingehall et al., 2017; Ruggiero et al., 2017; Kotfis et al., 2018; Racine et al., 2018; Shi et al., 2019; Boone et al., 2020; Cai et al., 2020).

So far, 10 studies have explored the correlation between OSA and POD. Four studies confirmed that OSA increased the risk of POD (Flink et al., 2012; Roggenbach et al., 2014; Nadler et al., 2017; Wu et al., 2022) while the remaining six did not identify their association (Gupta et al., 2001; Wang S. et al., 2018; Strutz et al., 2019; Tafelmeier et al., 2019; King et al., 2020; Oldham et al., 2021). Only three human studies and three animal experiments have explored the correlation between OSA and POCD. Two human studies showed that the incidence of POCD before discharge in OSA patients was lower than that of non-OSA patients (Wagner et al., 2018, 2021) and one human study showed that OSA patients had a higher risk of POCD in the early postoperative period than patients without OSA (Wu et al., 2022). Three animal models also suggested that the incidence of POCD was higher in OSA mice than that in non-OSA mice (Dong et al., 2018; Zhang et al., 2019; Mei et al., 2020). However, previous studies assessed cognitive function within 2 days after extubating with a lack of long-term postoperative follow-up cognitive assessments at 1-month or 1-year after surgery. In addition, no study, to our knowledge, has shown the effect of moderate-to-high risk of OSA combined with EDS on PND within 1 year after

surgery. Therefore, the first aim of our study was to explore the impact of moderate-to-high risk of OSA on the PND within 1 year postoperatively, and the second aim was to investigate the effect of moderate-to-high risk of OSA with EDS on PND within 1 year after surgery. We hypothesized that moderate-to-high risk of OSA with EDS was associated with the development of PND in older adults undergoing gastrointestinal surgery.

# Materials and methods

## Study design

With the ethical approval from the institutional review board of West China Hospital, this single-center prospective cohort study was conducted from June 4, 2019, to October 14, 2021, at a gastrointestinal surgical unit (≥80 beds, approximately 35 nurses, and 20 surgeons) of West China Hospital in Chengdu, China. All participants or their legal representatives provided written informed consent before the study. Patients did not receive financial compensation. Study methods and results are reported following the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement for prospective cohort studies (Vandenbroucke et al., 2007).

## Participants

We screened older adults (≥65 years) who were scheduled for stomach and intestinal surgery between June 4, 2019, and October 14, 2020. Patients who finally underwent the gastrointestinal procedure with at least a 3-day hospital stay, without communication barriers, and had a suitable condition for evaluation were included. The exclusion criteria are as follows: (1) cognitive impairment with a Mini-Mental State Examination (MMSE) scores less than 20; (2) delirium (assessed by Confusion Assessment Method-Severity (CAM-S Short Form) at baseline; (3) a documented history of severe psychosis such as severe depression, severe anxiety, autism, and schizophrenia; (4) a terminal condition with a life expectancy of fewer than 6 months (metastatic cancer, multiple organ failure, or receiving chemotherapy); (5) exploratory laparotomy without tumor removal or transferring to other departments after the operation; and (6) alcohol abuse or dependence within the last 3 months. Patients were also excluded if they had missing baseline, procedural, or outcome data (Figure 1).

## Assessments

The face-to-face evaluation was performed at the ward of the gastrointestinal surgery department on admission day by a researcher who received training in psychiatry and assessment methods. The baseline assessment included risks of OSA, subjective EDS, and objective napping. Then, the researcher and trained nurses assessed POD using the CAM-S twice daily (between 2–6 p.m. and 2–8 a.m.) until postoperative day seven or discharge. Another trained researcher blinded to the POD results performed postoperative cognitive assessments at discharge using MMSE and Telephone Interview for Cognitive Status-40 (TICS-40). Postoperative cognitive function at one month and one year after surgery were also evaluated using TICS-40 by telephone. The criteria for loss to follow-up were as follows: (1) refused to participate; (2) cannot be contacted; and (3) died (Figure 1).

## Exposure

The STOP-BANG questionnaire has good sensitivity and high diagnostic accuracy for detecting OSA in Asian populations (Chan et al., 2019). A STOP-BANG score of 3–8 indicates a moderate-to-high risk of OSA (OSA group) among the Chinese people, and a 0–2 score means a low risk of OSA (non-OSA group) (Chan et al., 2019). Subjective EDS was defined as an ESS score ≥ 11, and the Chinese version of ESS was a reliable and valid tool for measuring subjective EDS (Pak et al., 2017). Objective napping was measured using an Actiwatch-2 (Phillips Respironics Mini-Mitter), worn continuously on the non-dominant wrist for a minimum of two consecutive 24 h periods before operation. Participants also completed sleep logs for the period they wore the actigraphy and reported information regarding times they napped. Data for devices were collected in 1-min epochs and scored using Actiware software version 6.0.9 (Phillips Respironics, Bend, OR). Daily napping duration was calculated by summing up the time of napping periods throughout the day and averaging across all recording days. A nap ≥1 h/day during the day was considered objective napping (Leng et al., 2018). For convenience, subjective EDS and objective napping will be referred to as "EDS" throughout the rest of the manuscript.

## Cognitive function

The CAM-S provides a new delirium severity measure with strong psychometric properties and associations with important clinical outcomes. The Short Form includes the same four features as CAM: acute change or fluctuation, inattention, disorganized thinking, and altered level of consciousness. The diagnostic criteria of delirium are that the first and second criteria must be present, coming with the third or fourth criteria. The sum score of CAM-S Short Form ranges from 0 (no) to 7 (most severe) (Inouye et al., 2014). The Chinese version of the CAM-S demonstrated good reliability and validity in evaluating postoperative delirium among hospitalized Chinese geriatric patients (Mei et al., 2019). MMSE is scored from 0 to 30, with a test–retest reliability of 0.8–0.1 and a scorer reliability of 0.9–1.0. The Chinese-language version of the MMSE has been used in elderly Chinese, showing a high sensitivity of 87.6% and specificity of 80.8% (Li et al., 2016). MMSE is the instrument most widely used in screening for cognitive problems in hospitalized patients (Borchers et al., 2021). The Chinese version of the TICS-40 was previously validated and scored on a scale of 0 to 40, with a higher score indicating better cognitive function (Liu et al., 2021). A TICS-40 score ≤ 20 was defined as mild cognitive impairment, and a score ≤ 12 referred to dementia, according to a previous study (Wang et al., 2021). To control for learning effects (improvements over time with repeated testing), we applied an accepted approach that is using alternate forms of MMSE and TICS-40 (Munjir et al., 2015; Lee et al.,

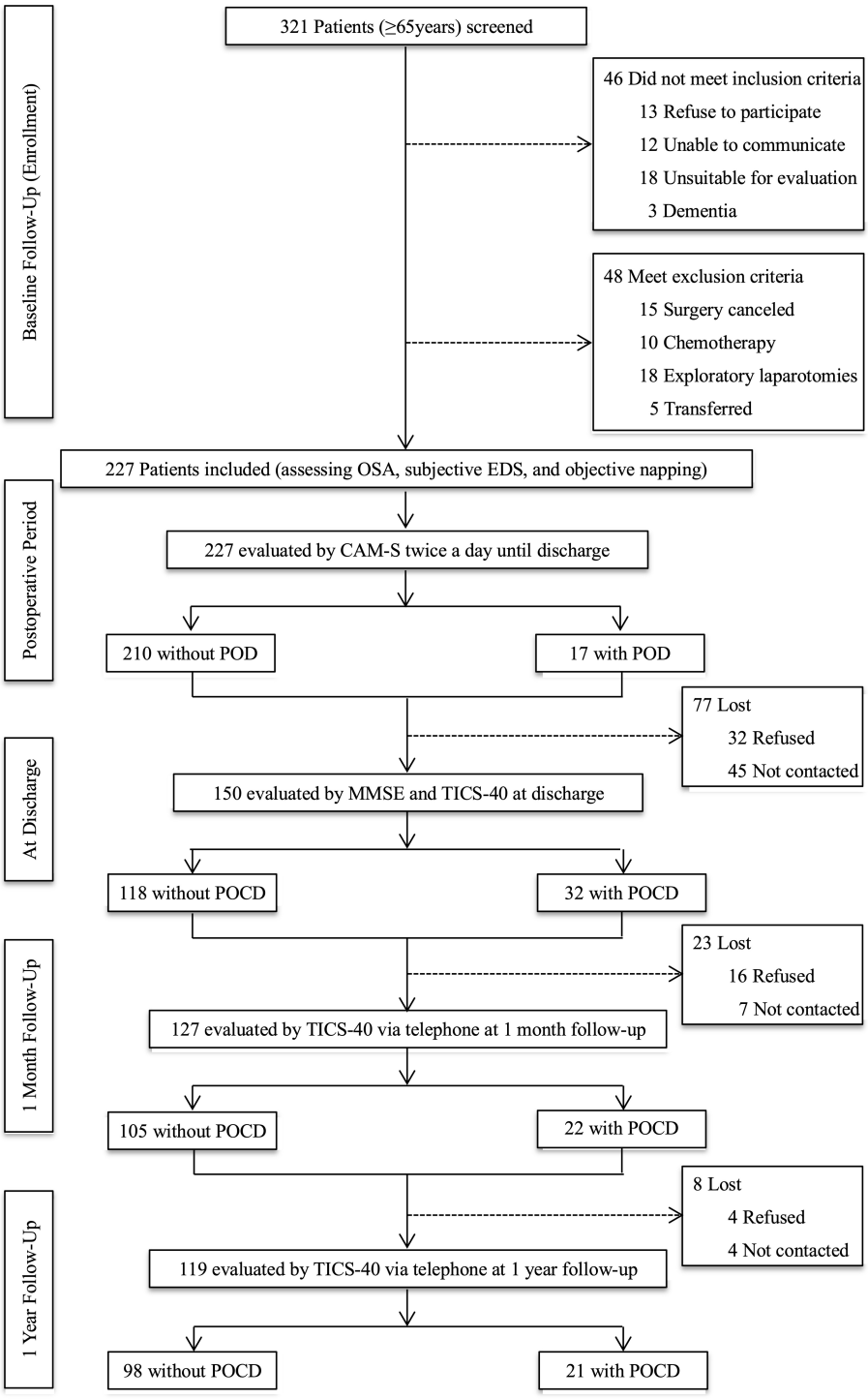


FIGURE 1
Study flow diagram. OSA, obstructive sleep apnea; EDS, excessive daytime sleepiness; CAM-S, Confusion Assessment Method-Severity; POD, postoperative delirium; MMSE, Mini-Mental State Examination; TICS-40, Telephone Interview for Cognitive Status-40; POCD, postoperative cognitive dysfunction.

2022). With the structures and scores unchanged, we changed the contents of questions at every postoperative cognition evaluation according to previous studies (e.g., "Please raise your hands," replaced the phrase, "Please close your eyes") (Katzman et al., 1988; Fong et al., 2009).

## Confounding factors

We also collected information on demographic characteristics, including age, sex, educational level, body mass index (BMI), smoking, drinking, exercise behavior, hypertension, and the American Society

of Anesthesiologists Physical Status Classification System (ASA). The Frail Scale (FS) has an excellent test–retest reliability of 0.7 in the Chinese community (Yuan et al., 2021). The total score of 5 items is 0 means health status, 1–2 means pre-frail, and 3–5 means frail (Yuan et al., 2021). Mini Nutritional Assessment-Short Form (MNA-SF) is a standard method to evaluate the nutritional status of the elderly. The Chinese version of MNA-SF performed well, and a score of 12–14 points is normal nutritional status, 8–11 points are at risk of malnutrition, and 0–7 points indicate malnutrition (Amasene et al., 2021; Yao et al., 2022). Furthermore, intraoperative data (operation time, anesthetic time, and general anesthesia method) (intravenous, inhalation, or a combination of both ways) were accessed from medical records.

## Outcomes

The primary outcomes were the incidence of PND, including POD during hospitalization, POCD at discharge, 1 month, and 1 year after surgery. Delirium appearing on one of all postoperative days before discharge was regarded as POD (Saczynski et al., 2014; Schmitt et al., 2015). The term POCD described in the literature ranged from within 24 h after surgery to 12 months (Evered and Silbert, 2018). In general, 1-month has been taken as a time in which the acute effect of surgery and anesthesia has abated (Evered and Silbert, 2018). POCD was mainly developed early after surgery and fully recovered cognitive function 3 months after surgery (Evered et al., 2018; Evered and Silbert, 2018). Few patients had cognitive decline persisting up to 1 year after surgery; this may indicate a possible progression to dementia (Evered et al., 2018). Therefore, early POCD was assessed at discharge and defined as at least one cognitive performance score (MMSE and the TICS-40 test) declined 2 points or more compared to the baseline score following previously used standards (Hollinger et al., 2021; Suraarunsumrit et al., 2022). POCD at 1 month and 1 year were defined as a decline in 1-month and 1-year TICS-40 performance of two points or more compared to preoperative TICS-40 score, respectively (Hollinger et al., 2021; Suraarunsumrit et al., 2022; van Zuylen et al., 2023).

## Statistical analysis

For univariate analysis, descriptive data were summarized using proportions for categorical data and means with standard deviation (SD) or medians with interquartile range (IQR) for continuous data. Comparison between PND and non-PND groups using $\chi^2$ analysis or Fisher exact probability test for categorical variables and *t*-test or, if the variables were not normally distributed, the Mann–Whitney test for continuous variables. The Wilcoxon rank sum tests were also used for ranked data. For multivariable analysis, we applied a multiple logistic regression model to adjust potential modifiers and estimate the effect of moderate-to-high risk of OSA, subjective EDS, and objective napping for PND. Furthermore, to examine the joint effect of moderate-to-high risk of OSA and EDS, we performed logistic regression models that included five dummy variables to represent all six possible combinations of moderate-to-high risk of OSA and EDS. We first used the moderate-to-high risk of OSA as a reference group, then we used no moderate-to-high risk of OSA with no subjective EDS and no objective napping as a reference group. All participants in our study used combined intravenous inhalational anesthesia, so the anesthetic method was not included in the regression model. Finally, confounding factors included age, BMI, FS score, MNA-SF score, and preoperative MMSE score in model 1; and confounding factors included age, BMI, FS score, MNA-SF score, preoperative MMSE, sex, and educational level in model 2. Statistical analysis and data visualization were performed using IBM SPSS, version 25 (IBM Corp, Armonk, NY, United States) and GraphPad Prism v.9 (GraphPad Software, San Diego, CA, United States). All tests were two-sided, and a $p$ value $< 0.05$ was designated as statistically significant.

# Results

## Characteristics of the study population based on PND categories

The descriptive statistics for the participants' perioperative characteristics according to the presence of PND are summarized in Table 1. A total of 227 participants (67.4% male; median (IQR) age: 69 (66–73) years) were included in the POD analyses, and 17 patients developed POD. Of 150 older adults (70.0% male; median (IQR) age: 69 (66–73) years) analyzed for POCD at discharge, 32 people had POCD. We finally included 127 (68.6% male; median (IQR) age: 69 (66–73) years) and 119 (63.9% male; median (IQR) age: 70 (67–75) years) patients for POCD at 1 month and 1 year analysis, respectively. The number of patients who developed POCD at 1 month was 22, and the cases of patients who existed POCD at 1 year were 21. The postoperative cognitive assessment results are shown in Supplementary Table S1. There was no significant difference among the PND (POD, POCD at discharge, POCD at 1 month, and POCD at 1 year) and non-PND groups in preoperative characteristic and surgical data except for the MNA-SF scores and MMSE scores. The MNA-SF scores were higher in the POD group than in the non-POD group. In addition, MMSE scores were lower in the POCD group compared to the non-POCD group at 1 year.

## Association between the moderate-to-high risk of OSA, subjective EDS, and objective napping

Correlations between the moderate-to-high risk of OSA, subjective EDS, and objective napping are shown in Table 2. The results (Table 2A) showed that subjective EDS was significantly associated with moderate-to-high risk of OSA and objective napping. However, as shown in Table 2B, patients with objective napping had a higher risk of OSA without statistical significance.

## Effect of the moderate-to-high risk of OSA, subjective EDS, or objective napping on PND

Univariate analysis was performed, and the results are shown in Supplementary Table S2. Multivariate analysis results in model 1 are

TABLE 1 Baseline characteristics of the study population by postoperative neurocognitive disorders categories.

| Characteristics | POD (*N* =227) | | | | POCD at discharge (*N* =150) | | | | POCD at 1 month (*N* =127) | | | | POCD at 1 year (*N* =119) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total (*N* =227) | No-POD (*n* =210) | POD (*n* =17) | *P*-value | Total (*N* =150) | No-POCD (*n* =118) | POCD (*n* =32) | *P*-value | Total (*N* =127) | No-POCD (*n* =105) | POCD (*n* =22) | *P*-value | Total (*N* =119) | No-POCD (*n* =98) | POCD (*n* =21) | *P*-value |
| Age, year | 69 (66–73) | 70 (67–73) | 69 (66–73) | 0.634 | 69 (66–73) | 68 (66–72) | 70 (66–73) | 0.466 | 69 (66–73) | 69 (66–72) | 70 (66–73) | 0.682 | 70 (67–75) | 69 (66–71) | 70 (67–75) | 0.357 |
| Male | 153 (67.4) | 141 (67.1) | 12 (70.6) | 0.771 | 105 (70.0) | 84 (71.2) | 21 (65.6) | 0.543 | 85 (66.5) | 72 (68.6) | 13 (59.1) | 0.390 | 76 (63.9) | 63 (64.3) | 13 (61.9) | 0.837 |
| Educational level[a] | | | | 0.506 | | | | 0.850 | | | | 0.144 | | | | 0.740 |
| Primary | 90 (39.6) | 83 (39.5) | 7 (41.2) | | 62 (41.3) | 50 (42.4) | 12 (37.5) | | 49 (38.6) | 37 (35.2) | 12 (54.5) | | 44 (37.0) | 35 (35.7) | 9 (42.9) | |
| Secondary | 106 (46.7) | 101 (48.1) | 5 (29.4) | | 65 (43.3) | 49 (41.5) | 16 (50.0) | | 57 (44.9) | 50 (47.6) | 7 (31.8) | | 57 (47.9) | 51 (52.0) | 6 (28.6) | |
| Higher | 31 (13.7) | 26 (12.4) | 5 (29.4) | | 23 (15.3) | 19 (16.1) | 4 (12.5) | | 21 (16.5) | 18 (17.1) | 3 (13.6) | | 18 (15.1) | 12 (12.2) | 6 (28.6) | |
| BMI | 22.7 ± 2.9 | 22.6 ± 3.0 | 23.4 ± 2.5 | 0.269 | 22.7 ± 3.1 | 22.6 ± 3.2 | 22.9 ± 2.7 | 0.639 | 22.6 ± 3.1 | 22.6 ± 3.2 | 22.6 ± 2.6 | 0.943 | 22.5 ± 2.8 | 22.3 ± 2.8 | 23.5 ± 2.5 | 0.087 |
| Smoke | 80 (35.2) | 76 (36.2) | 4 (23.5) | 0.293 | 57 (38.0) | 47 (39.8) | 10 (31.3) | 0.375 | 43 (33.9) | 37 (35.2) | 6 (27.3) | 0.473 | 35 (29.4) | 29 (29.6) | 6 (28.6) | 0.926 |
| Drink | 57 (25.1) | 53 (25.2) | 4 (23.5) | 0.876 | 42 (28.0) | 35 (29.7) | 7 (21.9) | 0.384 | 31 (24.4) | 26 (24.8) | 5 (22.7) | 0.840 | 25 (21.0) | 22 (22.4) | 3 (14.3) | 0.405 |
| Exercise | | | | 0.241 | | | | 0.855 | | | | 0.242 | | | | 0.976 |
| No | 32 (14.1) | 28 (13.3) | 4 (23.5) | | 23 (15.3) | 17 (14.4) | 6 (18.8) | | 18 (14.2) | 12 (11.4) | 6 (27.3) | | 17 (14.3) | 14 (14.3) | 3 (14.3) | |
| Occasionally | 11 (4.8) | 10 (4.8) | 1 (5.9) | | 9 (6.0) | 8 (6.8) | 1 (3.1) | | 7 (5.5) | 7 (6.7) | 0 (0.0) | | 6 (5.0) | 5 (5.1) | 1 (4.8) | |
| Every day | 184 (81.1) | 172 (81.9) | 12 (70.6) | | 118 (78.7) | 93 (78.8) | 25 (78.1) | | 102 (80.3) | 86 (81.9) | 16 (72.7) | | 96 (80.7) | 79 (80.6) | 17 (81.0) | |
| Hypertension | 116 (51.1) | 109 (51.9) | 7 (41.2) | 0.395 | 93 (62.0) | 71 (60.2) | 22 (68.8) | 0.375 | 76 (59.8) | 62 (59.0) | 14 (63.6) | 0.690 | 84 (70.6) | 70 (71.4) | 14 (66.7) | 0.664 |
| ASA status | | | | 0.204 | | | | 0.448 | | | | 0.692 | | | | 0.432 |
| I-II | 127 (55.9) | 115 (54.8) | 12 (70.6) | | 88 (58.7) | 67 (56.8) | 21 (65.6) | | 78 (61.4) | 65 (61.9) | 13 (59.1) | | 68 (57.1) | 54 (55.1) | 14 (66.7) | |
| III | 99 (43.6) | 94 (44.8) | 5 (29.4) | | 61 (40.7) | 51 (43.2) | 10 (31.3) | | 48 (37.8) | 40 (38.1) | 8 (36.4) | | 50 (42.0) | 44 (44.9) | 6 (28.6) | |
| IV | 1 (0.4) | 1 (0.5) | 0 (0.0) | | 1 (0.7) | 0 (0.0) | 1 (3.1) | | 1 (0.8) | 0 (0.0) | 1 (4.5) | | 1 (0.8) | 0 (0) | 1 (4.8) | |
| FS score | 0 (0–1) | 0 (0–1) | 0 (0–1) | 0.824 | 0 (0–1) | 0 (0–1) | 0 (0–1) | 0.306 | 0 (0–1) | 0 (0–1) | 0 (0–1) | 0.370 | 0 (0–1) | 0 (0–1) | 0 (0–1) | 0.574 |
| MNA-SF scores | 12 (10–13) | 13 (11–14) | 11 (10–13) | **0.007** | 12 (10–13) | 12 (10–13) | 12 (10–13) | 0.893 | 12 (10–13) | 12 (10–13) | 12 (10–13) | 0.938 | 12 (10–13) | 12 (10–13) | 12 (10–13) | 0.169 |
| MMSE scores | 29 (27–30) | 29 (27–30) | 28 (27–29) | 0.542 | 29 (27–30) | 29 (27–30) | 29 (27–29) | 0.181 | 29 (27–30) | 29 (26–30) | 29 (28–30) | 0.454 | 29 (27–30) | 29 (28–30) | 28 (27–29) | **0.017** |
| TICS-40 scores | 33 (32–34) | 33 (32–34) | 33 (32–34) | 0.214 | 33 (32–34) | 33 (32–34) | 33 (32–34) | 0.735 | 33 (32–34) | 33 (32–34) | 33 (31–34) | 0.671 | 33 (31–34) | 33 (31–34) | 33 (32–34) | 0.297 |
| Operation times, h[b] | 2.8 ± 1.1 | 2.7 ± 1.1 | 3.2 ± 1.0 | 0.117 | 2.8 ± 1.1 | 2.8 ± 1.1 | 2.7 ± 1.2 | 0.566 | 2.7 ± 1.0 | 2.7 ± 1.0 | 2.8 ± 1.0 | 0.657 | 2.7 ± 1.1 | 2.8 ± 1.1 | 2.5 ± 1.1 | 0.313 |
| Anesthetic time, h[c] | 4.0 ± 1.3 | 4.0 ± 1.3 | 4.2 ± 1.0 | 0.617 | 4.1 ± 1.3 | 4.1 ± 1.2 | 4.2 ± 1.6 | 0.706 | 4.0 ± 1.2 | 4.0 ± 1.2 | 4.2 ± 1.3 | 0.460 | 4.0 ± 1.3 | 4.0 ± 1.2 | 4.0 ± 1.5 | 0.787 |

The normally distributed variables are presented as mean ± SD, non-normally distributed variables are presented as median (IQR), the rank variables and categories variables are presented as frequency (%). The normally distributed data were compared using unpaired 2-tailed *t*-test, the non-normally distributed data and rank data were compared with Mann–Whitney U test, and the categories data were compared using the Pearson chi-square test. The bold values indicated that $p$ value <0.05.

[a]Primary educational level included an illiterate or primary school diploma; secondary educational level consisted of a junior high school, senior high school, or technical secondary school diploma; high educational level meant having a college, bachelor, master's degree or above.

[b]Operation time started from skin discission to incision close except closed reduction.

[c]Anesthetic time started from the injection of anesthetic until the patient woke up.

POD, postoperative delirium; POCD, postoperative cognitive dysfunction; BMI, body mass index; ASA, American Society of Anesthesiologists; FS, frail scale; MNA-SF, mini nutritional assessment-short form; MMSE, Mini-Mental State Examination; TICS-40, Telephone Interview for Cognitive Status-40; SD, standard deviation; IQR, interquartile range.



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TABLE 2 (A) Association between the moderate-to-high risk of OSA, subjective EDS, and objective napping. (B) Association between the moderate-to-high risk of OSA and objective napping.

| A | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| OSA | POD (*N* =227) | | | POCD at discharge (*N* =150) | | | POCD at 1 month (*N* =127) | | | POCD at 1 year (*N* =119) | | |
| | No-EDS (*n* =179) | EDS [c] (*n* =48) | *P*-value | No-EDS (*n* =118) | EDS (*n* =32) | *P*-value | No-EDS (*n* =99) | EDS (*n* =28) | *P*-value | No-EDS (*n* =94) | EDS (*n* =25) | *P*-value |
| Moderate-to-high risk of OSA[a] | 116 (64.8) | 47 (97.9) | **<0.001** | 84 (71.2) | 31 (96.9) | **0.002** | 67 (67.7) | 27 (96.4) | **0.002** | 56 (59.6) | 25 (100) | **<0.001** |
| Objective napping[b] | 68 (38.0) | 27 (56.3) | **0.023** | 42 (35.6) | 19 (59.4) | **0.015** | 33 (33.3) | 16 (57.1) | **0.022** | 33 (35.1) | 12 (48.0) | 0.237 |

| B | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| OSA | POD (*N* =227) | | | POCD at discharge (*N* =150) | | | POCD at 1 month (*N* =127) | | | POCD at 1 year (*N* =119) | | |
| | No-naps (*n* =132) | Naps (*n* =95) | *P*-value | No-naps (*n* =89) | Naps (*n* =61) | *P*-value | No-naps (*n* =78) | Naps (*n* =49) | *P*-value | No-naps (*n* =74) | Naps (*n* =45) | *P*-value |
| Moderate-to-high risk of OSA | 94 (71.2) | 69 (72.6) | 0.815 | 67 (75.3) | 48 (78.7) | 0.628 | 57 (73.1) | 37 (75.5) | 0.761 | 50 (67.6) | 31 (68.9) | 0.881 |

The categories variables were presented as frequency (%) and compared using the Pearson chi-square test, or when at least one of the cells contingency tables had an expected $n < 5$, the Fisher exact probability test. The rank data were compared with the Mann–Whitney U test. The bold values indicated that $p$ value <0.05.

OSA, obstructive sleep apnea; EDS, excessive daytime sleepiness; POD, postoperative delirium; POCD, postoperative cognitive dysfunction; ESS, Epworth sleepiness scale.

[a]Moderate-to-high risk of OSA defined as a STOP-BANG score of 3–8.

[b]Objective napping is considered as sleep time of more than 60 min between 9 a.m. and 7 p.m.

[c]EDS meant subjective EDS and was defined as an ESS score > 10.

shown in Figure 2, and multivariate analysis results in model 2 are shown in Supplementary Figure S1.

In multivariate logistic regression analysis for PND, patients who reported subjective EDS had nearly three times (OR, 2.82; 95% CI, 1.09–7.29; $p = 0.033$) as likely to develop POCD at discharge compared with patients without subjective EDS. The participants who had objective napping also had almost three times (OR, 3.09; 95% CI, 1.28–7.47; $p = 0.012$) as likely to develop POCD at discharge compared with those without objective napping. In addition, the incidence of POCD at 1 month after surgery significantly increased by 294% (OR, 3.94; 95% CI, 1.26–12.36; $p = 0.019$) among patients with objective napping than those without napping. Objective napping also caused a 677% (OR, 7.77; 95%CI, 2.33–25.89; $p = 0.001$) increase in the risk of POCD at 1 year after surgery.

However, patients with moderate-to-high risk of OSA had a slightly higher incidence of POD (OR, 1.26; 95% CI, 0.29–5.40; $p = 0.759$), POCD at discharge (OR, 1.74; 95% CI, 0.56–5.45; $p = 0.342$), 1 month (OR, 1.29; 95% CI, 0.52–3.19; $p = 0.577$), and 1 year (OR, 1.42; 95% CI, 0.36–5.66; $p = 0.621$) after surgery compared with patients with low risk of OSA, which were not statistically significant differences.

## Moderate-to-high risk of OSA combined with subjective EDS or objective napping was associated with PND

The results of the univariate analysis are shown in Supplementary Table S2. Multivariate analysis results in model 1 are shown in Figure 3, and multivariate analysis results in model 2 are shown in Supplementary Figure S2.

Patients with moderate-to-high risk of OSA and subjective EDS showed an OR of 5.42 (95% CI, 1.11–26.38; $p = 0.037$) for POCD developing at discharge compared with patients only having a moderate-to-high risk of OSA. Patients with both moderate-to-high risk of OSA and subjective EDS also presented an OR of 8.00 (95% CI, 1.12–57.28; $p = 0.038$) for risk of POCD at discharge compared with patients without moderate-to-high risk of OSA, subjective EDS, and objective napping.

Patients with moderate-to-high risk of OSA and objective napping showed an OR of 4.69 (95% CI, 1.33–16.52; $p = 0.016$) for POCD at discharge, an OR of 6.75 (95% CI, 1.40–32.62; $p = 0.018$) for POCD at 1-month, and an OR of 6.41 (95% CI, 1.15–35.87; $p = 0.035$) for POCD at 1-year after surgery compared with patients with moderate-to-high risk of OSA alone, respectively. Also, patients with both

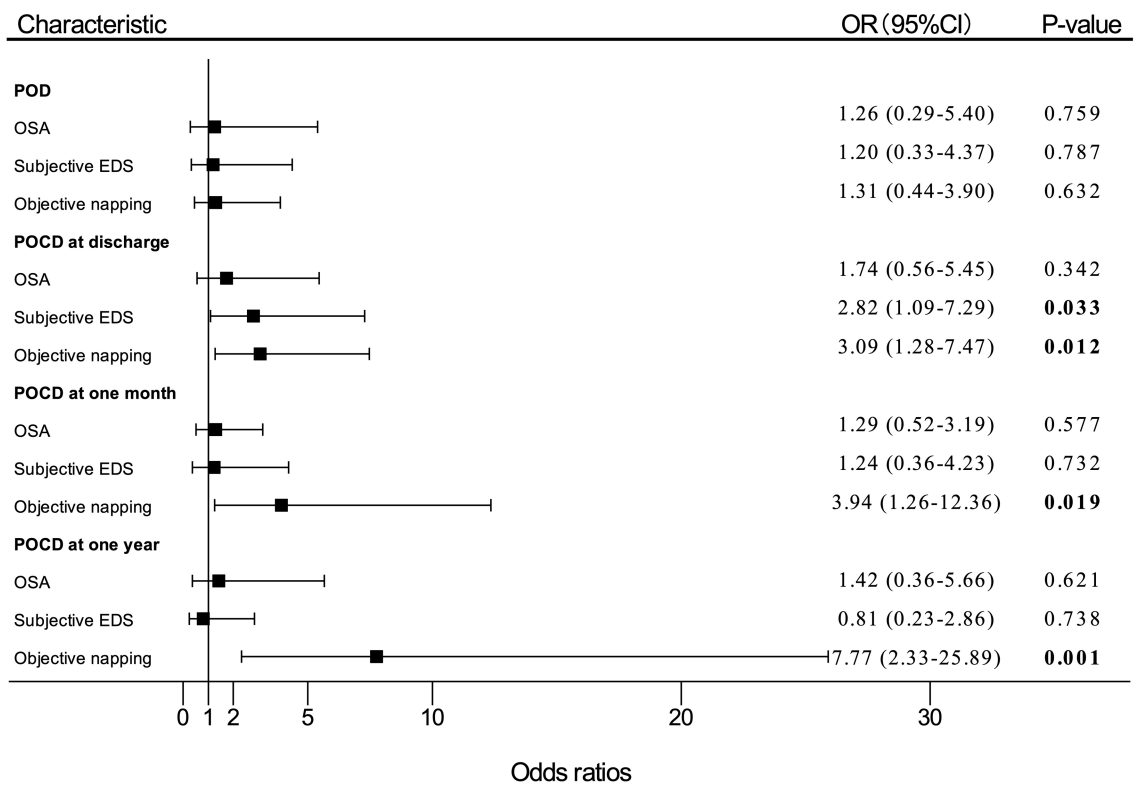


FIGURE 2
Adjusted (OR) and 95%CI of the effect of OSA or EDS on PND. Estimates were obtained by logistic regression, after adjusting for the significant terms: age, BMI, FS score, MNA-SF score, and MMSE score. "OSA" means patients with moderate-to-high risk of OSA. OR, odds ratio; OSA, obstructive sleep apnea; EDS, excessive daytime sleepiness; PND, perioperative neurocognitive disorders; BMI, body mass index; FS, frail scale; MNA-SF, mini nutritional assessment-short form; MMSE, Mini-Mental State Examination; POD, postoperative delirium; POCD, postoperative cognitive dysfunction.

moderate-to-high risk of OSA and objective napping presented an OR of 6.92 (95% CI, 1.09–43.97; $p = 0.040$) for POCD at discharge, an OR of 12.97 (95% CI, 1.50–112.19; $p = 0.020$) for POCD at 1-month, and an OR of 15.20 (95% CI, 1.25–185.13; $p = 0.033$) for POCD at 1-year after surgery compared patients without moderate-to-high risk of OSA, subjective EDS, and objective napping, respectively.

## Discussion

To our knowledge, this is the first prospective cohort study to explore whether the moderate-to-high risk of OSA and moderate-to-high risk of OSA combined with EDS are associated with PND. We found that patients with moderate-to-high risk of OSA had a slightly higher incidence of PND than those with low-risk OSA, which were not statistically significant differences. However, moderate-to-high risk of OSA combined with subjective EDS significantly increased the risk of POCD at discharge. Furthermore, moderate-to-high risk of OSA combined with objective napping increased the risk of POCD at discharge, 1 month, and 1 year after surgery.

Our results did not show a significant association between moderate-to-high risk of OSA and POD, which were consistent with three studies that found no association between high preoperative risk of OSA assessed by the STOP-BANG questionnaire and the incidence of POD (Wang S. et al., 2018; King et al., 2020). However, there were four cohort studies suggested that a higher apnea-hypopnea index will increase the risk of POD development (Flink et al., 2012; Roggenbach et al., 2014; Nadler et al., 2017; Wu et al., 2022). The inconsistency of the results may be due to the limitation of the STOP-BANG questionnaire to distinguish the severity of OSA. Previous studies showed that the STOP-BANG had consistently high levels of sensitivity and low levels of specificity when compared to polysomnography (PSG) regardless of the patient population (Miller et al., 2018). Therefore, some patients with a low risk of OSA were wrongly diagnosed with a moderate-to-high risk of OSA, which caused the difference in POD between OSA and non-OSA groups to be not obvious. Besides, the CAM-S short form used by non-psychiatrists to detect POD will have relatively low sensitivity (Inouye et al., 2014). POD occurring at other times may be overlooked except for the evaluation time. Then, the incidence of POD in the OSA group was 8% in our study, which was much lower than the 53% and 44% reported by previous research (Flink et al., 2012; King et al., 2020). Our study may lack the power to detect this small difference in POD between the two groups to some extent. However, it is also necessary to formally implement preoperative assessment of the STOP-BANG questionnaire into the standard for POD risk stratification regarding patients undergoing surgery who do not undergo routine PSG. Because PSG, which with high expense, relative inaccessibility, and time consumption, can delay the diagnosis and treatment of OSA. Future studies are needed to explore the association between OSA and POD and clarify the relevance of preoperative OSA

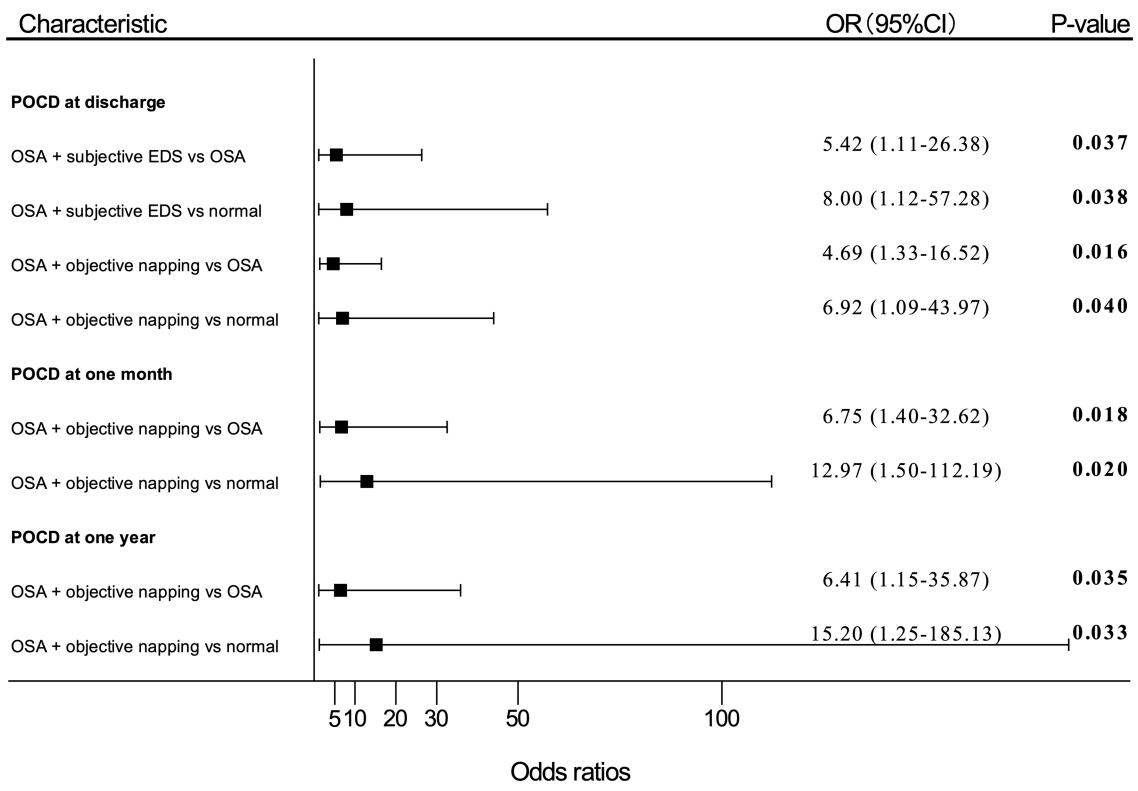


FIGURE 3
Adjusted (OR) and 95%CI of the effect of OSA with EDS on PND. Estimates were obtained by logistic regression, after adjusting for the significant terms: age, BMI, FS score, MNA-SF score, and MMSE score. "OSA" means patients with moderate-to-high risk of OSA; "normal" means patients with no OSA, no subjective EDS, and no objective napping. OR, odds ratio; OSA, obstructive sleep apnea; EDS, excessive daytime sleepiness; PND, perioperative neurocognitive disorders; BMI, body mass index; FS, frail scale; MNA-SF, mini nutritional assessment-short form; MMSE, Mini-Mental State Examination; POD, postoperative delirium; POCD, postoperative cognitive dysfunction.

and the accuracy of the STOP-BANG questionnaire when used for POD risk stratification.

Our study firstly assessed the effect of moderate-to-high risk of OSA on the risk of POCD at discharge, 1 month, and 1 year after surgery, and the damaging effect of OSA on POCD during 1-year follow-up periods has no statistical significance. However, one clinical trial retrospectively analyzed clinical data and found that OSA diagnosed by PSG may adversely affect postoperative cognitive function before discharge (Wu et al., 2022). In addition to the limitations of the STOP-BANG questionnaire to identify OSA risk, the insensitivity of questionnaires we used to detect mild cognitive impairment may cause difficulty in recognizing the slight differences between the two groups. Recommendations for the measurement of POCD are neuropsychological tests, usually in the form of multiple tests administered as a test battery (Evered et al., 2018). Another explanation is that the incidence of POCD depends on the type of surgery and surgical populations. Patients undergoing joint replacement are more prone to PND than patients with gastrointestinal surgery, (Postler et al., 2011; Norkienė et al., 2013; Krenk et al., 2014; Aceto et al., 2015; Goettel et al., 2017; Kotfis et al., 2018; Wang C. G. et al., 2018; Chaiwat et al., 2019; Guo et al., 2020; Kang et al., 2020; Wang et al., 2020; Yang et al., 2020; Li et al., 2021) and our study may need more sample size to detect differences in PND between the two groups. Furthermore, previous mouse models also confirmed that chronic intermittent hypoxia could worsen cognitive performance during the first 4 days after surgery (Dong et al., 2018; Zhang et al., 2019; Mei et al., 2020). The advantage of animal models is that they permit a consistent, often severe, level of risk exposure and complete mitigation. Therefore, the presence of OSA may play an important role in POCD in clinical practice, and the relationship between OSA and POCD within 1 year after surgery warrants future exploration in a prospective cohort study with a large sample size and the gold standard for OSA and POCD should be used.

Our study found that moderate-to-high risk of OSA with subjective EDS was associated with POCD at discharge, and moderate-to-high risk of OSA with objective napping was a predictor for POCD at discharge, 1 month, and 1 year after surgery. The mechanism of why the moderate-to-high risk of OSA with EDS may increase the incidence of PND is unclear. Only three animal experiments found that POCD in rats exposed to chronic intermittent hypoxia might be attributed to neuroinflammation (marked by microglial activation and IL-1β levels) (Dong et al., 2018; Zhang et al., 2019; Mei et al., 2020). Significantly, one study involving 58 OSA patients suggested that OSA with objective EDS was the more severe phenotype of the disorder associated with low-grade inflammation (Li et al., 2017). Another clinical study found that EDS might be a potentially useful clinical marker to identify patients with severe OSA at risk of metabolic syndrome (Huang et al., 2016). A recent study indicated

that subjective EDS in OSA may be related to neuronal injury and disruptions in the dopaminergic system (Paik et al., 2014). Neuroimaging studies of brain structure also have resulted in a consensus that white matter, gray matter, and hippocampal damage are present in patients with OSA and EDS (Lal et al., 2021). In summary, OSA with EDS represents a severe phenotype and is identified as an important contributor to poor outcomes. Surgical stress can further aggravate inflammation and metabolic disorder and accelerate neuronal injury/apoptosis, ultimately causing cognitive impairment. Importantly, these findings emphasize the need for clinicians to pay particular attention to OSA in combination with EDS in elderly patients, which may impact the development of PND and clinical decision-making regarding treatment. Furthermore, the results of this study do not support the use of OSA alone to make individual treatment decisions, but the combined use of the STOP-BANG questionnaire and subjective EDS/ longer objective napping to predict PND.

Another important thing to note is that, in our study, moderate-to-high risk of OSA with subjective EDS only predicted POCD at discharge. But the moderate-to-high risk of OSA with objective naps predicted POCD at discharge, 1 month, and 1 year after surgery. The reason might be that subjective EDS only reflect perceived sleepiness but is less likely to capture unplanned naps among older adults, which could lead to underestimation of napping behaviors. One study showed that older adults rarely reported EDS and did not always recognize napping or how much they napped (McPhillips et al., 2020). Some studies have compared the effect of objective daytime naps and subjective EDS on cognitive dysfunction (Bolitho et al., 2013; Gotts et al., 2015). The findings suggested that daytime actigraphy, a non-invasive and inexpensive objective measure of daytime sleep, could predict patients with cognitive dysfunction rather than subjective EDS. In addition, subjective EDS and objective napping may reflect two different central nervous system processes. The ESS captures the subjective complaint of daytime sleepiness resulting from impaired sustained attention (Yun et al., 2015) whereas longer daytime nap is associated with an increased level of inflammation or abnormal brain metabolites (Spira et al., 2018). This hypothesis is supported by several studies indicating that objective, but not subjective, sleepiness is associated with inflammation in patients with OSA (Li et al., 2017; Mehra et al., 2017). Therefore, objective napping, compared to subjective EDS, is a better predictor of daytime impairment and PND risks. There is a need to use daytime naps in the routine evaluation of older adults with OSA before surgery.

This study has several limitations. Firstly, we evaluated OSA using STOP-BANG but not polysomnography, which may underestimate the severity of OSA, especially in the presence of high AHI values. The association between preoperative OSA, especially severe OSA combined with EDS, and PND may be greater than what we observed. Secondly, our study used actigraphy to obtain objective daytime naps, but MSLT is considered the standard gold method for the objective measure of daytime sleepiness. It is unknown if actigraphy has the same effect as MSLT for measuring objective EDS. However, given that MSLT is a cumbersome and expensive measure of EDS, there is a need to validate easy-to-use and inexpensive methods of objective EDS to be used in the routine evaluation of OSA patients. This work provided efference to actigraphy applications in older adults with OSA before surgery. Thirdly, we did not use a neuropsychological test battery, considered the gold standard for PND diagnosis, to assess PND. In addition, we did not use the Montreal Cognitive Assessment (MoCA), which has a higher sensitivity than MMSE among elderly patients, to evaluate cognitive performance at discharge. The limitation of measuring tools could significantly underestimate the number of patients with PND. The association between OSA, EDS, or OSA with EDS and PND may exist undetected. Fourthly, while we controlled for learning effects of MMSE and TICS, patients recovered above baseline levels at 1 year suggesting either that this control was incomplete, or that patients had depressed cognitive levels at baseline likely due to pain, pre-admission narcotics, other psychoactive medications, or immobility. Fifthly, the incidence of POD in our study is 7%, which is lower than the rate of POD reported in previous studies. Our study may lack the power to detect this small difference in POD between the two groups to some extent. Sixthly, many factors, including postoperative pain, anxiety, and discomfort, may mediate the association between sleep and long-term cognitive outcomes. While we wanted to avoid over-controlling for variables that might be intermediaries between sleep and cognitive decline, such control will be important in future work to clarify mechanisms and targets for intervention. Finally, our study has some degree of loss to follow-up despite our efforts to assess all participants. The rate of loss to follow-up at discharge, 1-month and 1-year after surgery were similar in both OSA and non-OSA groups, and there was no difference in baseline data between patients lost to follow-up and those lost to follow-up. Thus, our results are reasonable and believable, assuming nondifferential misclassification.

In conclusion, our study suggested that preoperative moderate-to-high risk of OSA combined with EDS could predict a higher risk of POCD at discharge, 1 month, and 1 year after surgery. However, moderate-to-high risk of OSA alone could not precit the development of PND within 1 year after surgery. Given that our study is the first to examine the role of moderate-to-high risk of OSA and moderate-to-high risk of OSA with EDS on the development of PND, more research is needed to explore the effect of OSA and OSA with EDS on PND in independent prospective cohorts. Furthermore, our results suggest that measures of moderate-to-high risk of OSA and EDS are of clinical utility in the identification of high-risk PND in the elderly. Preventions and treatments against OSA and EDS should be investigated and implied to maintain cognitive functional capacity in elderly patients within 1 year after surgery.

## Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary material.

## Ethics statement

The studies involving human participants were reviewed and approved by the ethical approval from the institutional review board of West China Hospital. The patients/participants provided their written informed consent to participate in this study.

## Author contributions

WW and XH initiated the idea for this article and prepared the final copy of the manuscript. GW is responsible for taking pictures and tables. WW and YW took responsibility for collecting patient data. LP and QC took responsibility for reviewing this article. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



## Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2023.1161279/full#supplementary-material

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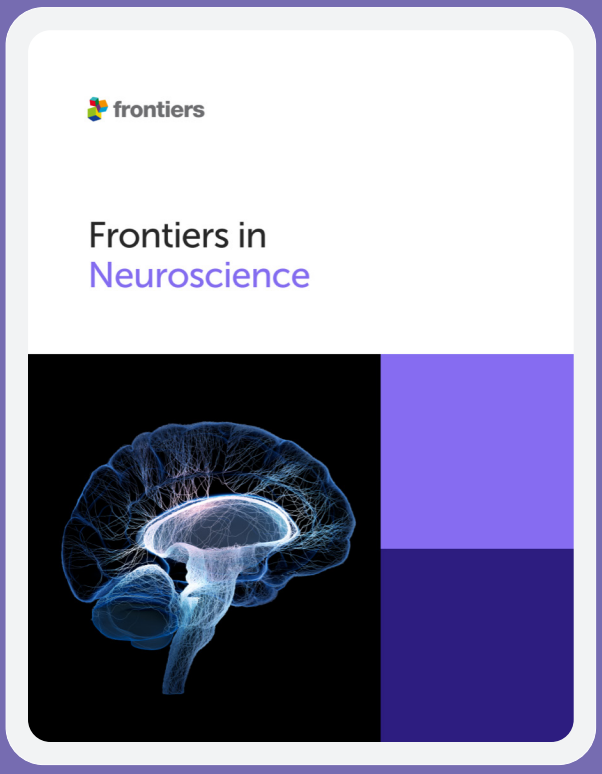


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