EEG AND fMRI FOR SLEEP AND SLEEP DISORDERS - MECHANISMS AND CLINICAL IMPLICATIONS

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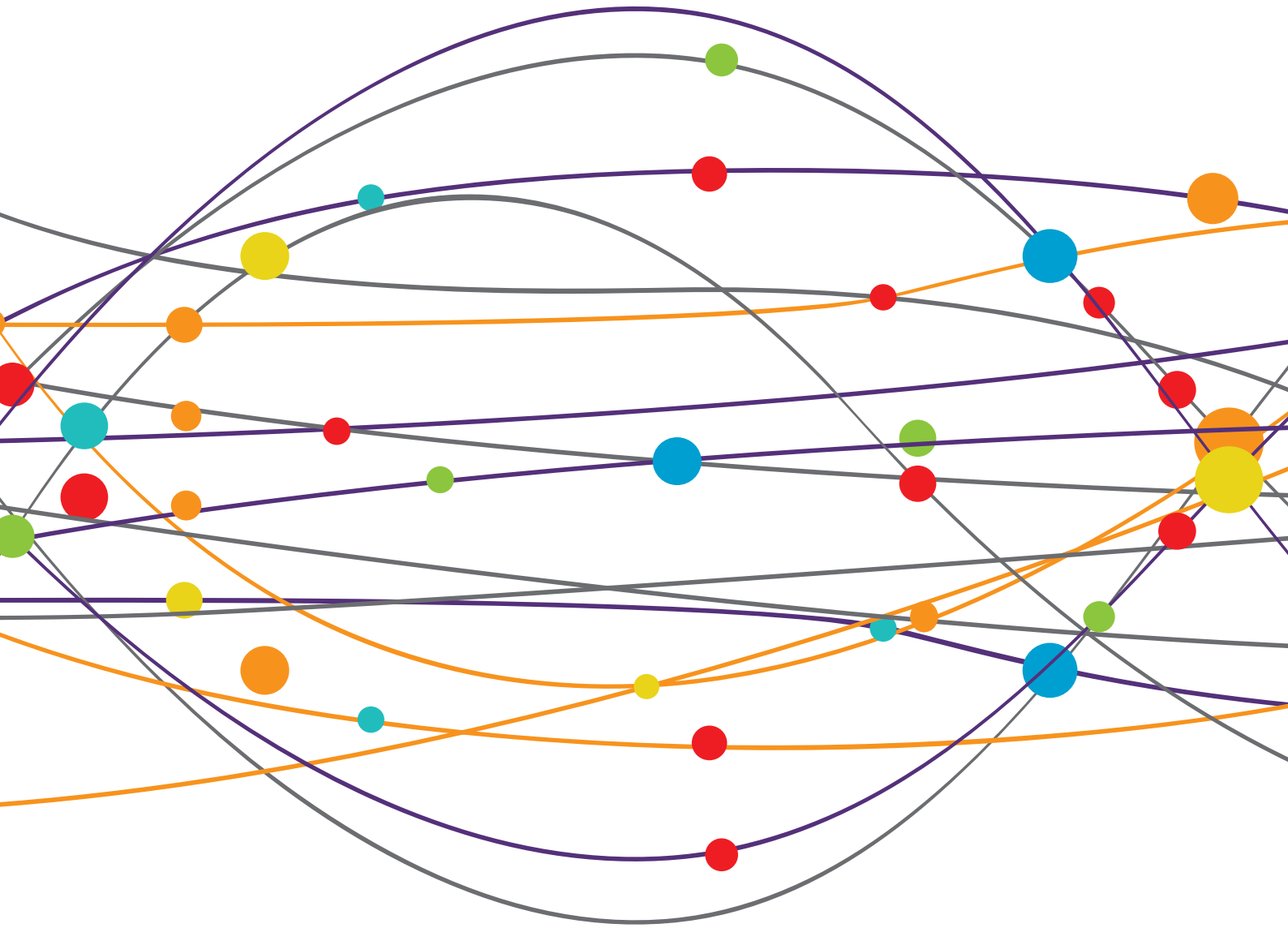

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EEG AND fMRI FOR SLEEP AND SLEEP DISORDERS - MECHANISMS AND CLINICAL IMPLICATIONS

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EDITORIAL
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Editorial: EEG and fMRI for Sleep and Sleep Disorders–Mechanisms and Clinical Implications

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Keywords: EEG, functional magnetic resonance imaging, sleep disorders, sleep deprivation, obstructive sleep apnea, shift work, sleepiness, restless legs syndrome

Editorial on the Research Topic

EEG and fMRI for Sleep and Sleep Disorders - Mechanisms and Clinical Implications

INTRODUCTION

In modern society, more and more people undergo an increased incidence of sleep disorders. Sleep disorders are a hallmark of most neurological diseases, yet sleep disorders themselves are commonly found to have far more health impact than previously thought. While it is generally accepted that sleep is critical for normal brain function, there is as yet no agreement upon the function of sleep. Although there has been a surprising upsurge in neuroimaging findings that address the brain's structural and functional changes associated with sleep disorders, sleep is less frequently studied using imaging techniques than many neuropsychiatric disorders (1). Thus, modern sleep medicine requires a multi-modal approach to investigate brain changes occurring in response to or as causative mechanisms of sleep-related neuropathology.

Electroencephalography (EEG) and functional magnetic resonance imaging (fMRI) are key neuroimaging techniques used to explore the mechanisms of sleep disorders. Sleep spindle, K-complex, and sleep slow wave are specific sleep electrical waves, which are very important for us to understand the mechanisms and clinical implications of sleep. Clinically, human sleep and sleep pathology are defined based upon scalp recorded electrical potentials (EEG), which have provided seminal information on the changes of brain electrophysiology that are associated with sleep pathologies and pathologies that adversely affect sleep. In this issue, EEG and fMRI have been utilized to examine changes associated with pathologies of sleep. The aim of this Research Topic is to contribute to a better understanding of the etiology of sleep disorders.

SLEEP DEPRIVATION

This specific issue includes two studies focusing on sleep deprivation (SD). In one study by Zeng et al., the authors reported regional brain activity alteration in healthy subjects after a total of 36 h of SD relative to after normal sleep, using a percent amplitude of fluctuation (PerAF) method. They found that SD resulted in a 2.23% decrease in accuracy rate and an 8.82% increase in reaction time, and the SD was associated with decreased PerAF in the bilateral dorsolateral prefrontal cortex, which positively correlated with the accuracy rate.

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In the second study by Li et al., these authors examined the association of the thalamus with SD-related emotion changes. In their study, positive emotions and psychomotor performance were reduced, while negative emotions were increased after a total of 36 h of SD. The left thalamus was selected as a region of interest for seed point functional connectivity, and decreased functional connectivity was found between this area and left middle temporal gyrus, left inferior frontal gyrus, right thalamus, right inferior temporal gyrus, left middle temporal pole gyrus, right calcarine, left cuneus, left rectus, and left medial superior frontal gyrus. Among these connectivity pairs, the decreased functional connectivity between the left thalamus and the left inferior frontal gyrus negatively correlated with negative emotion changes.

OBSTRUCTIVE SLEEP APNEA

Two studies focusing on obstructive sleep apnea were included in our specific issue. The first study by Zhou et al. examined functional connectivity changes in the bilateral hippocampus in treatment-naïve patients with moderate or severe obstructive sleep apnea. It was found that extensive abnormal functional connectivity of the right hippocampus may contribute to the joint effect of intermittent nocturnal hypoxia and sleep fragmentation, while the reduced functional connectivity between the left hippocampus and the anterior cerebellar lobe was more likely caused by a disturbed sleep architecture.

The second study by Wu Y. et al. summarizes the literature on resting-state EEG and resting-state fMRI (rs-fMRI) studies in patients with obstructive sleep apnea. Evidence from EEG-related studies showed that patients with obstructive sleep apnea were associated with increased power of δ and θ in the frontal and central regions, and their slow-wave activity showed a positive correlation with their apnea-hypopnea index. For rs-fMRI studies, the prefrontal cortex and insula may be the vital regions of obstructive sleep apnea, and these areas strongly correlated with the severity of diseases. Furthermore, some large-scale brain networks, such as the default-mode network, salience network, and central executive network, play important roles in the pathology of obstructive sleep apnea. Finally, they proposed four suggestions about the intervention therapy for obstructive sleep apnea.

NARCOLEPSY/SLEEPINESS

In this specific issue, three studies focusing on narcolepsy/sleepiness-related issues were included. One study by Bioulac et al. found drug-free sleepy patients with attention deficit hyperactivity disorder (ADHD) had significantly shorter sleep latency relative to normal controls during the maintenance of wakefulness test, and the difficulty to remain awake during soporific circumstances observed in these patients with ADHD cannot be explained by changes in the kinetic of sleep pressure buildup.

The second study by Deng et al. investigated the dynamic cerebral autoregulation, as assessed by phase difference using transfer function analysis, in patients with central disorders of hypersomnolence. The study found that these patients were associated with impaired dynamic cerebral autoregulation and that recovery is possible after treatment in patients with narcolepsy type 1.

The third study by Zhu et al. applied simultaneous EEG-fMRI to examine the brain network topological characteristics in patients with narcolepsy type 1 (NT1) disorder. The study found lower global brain network efficiency during the N2 sleep stage and reduced small-world attributes in NT1 patients, relative to normal controls.

SHIFT WORK

Two studies examined brain network changes caused by shift work. One study by Ning et al. first identified seven brain networks and evaluated their granger causality connections. The study reported increased inflows from the anterior default mode network (aDMN) to sensorimotor network (SMN) and left frontoparietal network (LFPN) to salience network (SN), and negative associations with attention and visuospatial scores. In another study by Wu X. et al., it was found that night shift work could lead to poor sleep quality and lower melatonin levels, as well as a series of changes in regional brain activity and connectivity.

RESTLESS LEGS SYNDROME

One study by Sun et al. evaluated the sleep characteristics and their related risk factors among Parkinson's disease (PD) patients with and without restless legs syndrome (RLS). This study provided evidence that PD patients with RLS exhibited worse nocturnal sleep than PD patients without RLS. They also observed several risk factors, such as periodic limb movements during sleep in PD patients with RLS.

SLEEP IN PATIENTS WITH PTSD

One study by Laxminarayan et al. compared spectral power and synchrony features in subjects with post-traumatic stress disorder (PTSD) during two consecutive nights of sleep. They developed a classifier that could be used to discriminate subjects with and without PTSD using robust stage-independent and whole-night features from EEG signals.

CONCLUSIONS

Together, this issue features articles that address the relationships between sleep disorders and changes in brain electrophysiology, structure, and function using EEG and neuroimaging methods. We hope this issue will provide seminal information for a better understanding of how sleep disorders affect our brain and behavior. We believe that this issue will stimulate discussions beyond those

working in the field, in particular in those patients suffering from sleep disorders. Public health guidance should pay more attention to sleep problems and advocate healthy lifestyle habits.

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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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ORIGINAL RESEARCH
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Assessing Cognitive Abilities of Patients With Shift Work Disorder: Insights From RBANS and Granger Causality Connections Among Resting-State Networks

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Background: Numerous studies have confirmed that long-term shift work is not only associated with increased health problems and acute impact on safety but also with impaired cognitive abilities. However, very little is known about effects of shift work on cognition-related brain resting-state networks. The aim of this study was to explore the effects of shift work disorder (SWD) on granger causality connection among resting-state brain networks.

Methods: Thirty patients with SWD and 25 matched healthy subjects were recruited to undergo the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) and resting-state fMRI scanning. We employed independent component analysis (ICA) to extract resting-state brain networks and granger causality analysis (GCA) to characterize the difference of granger causality connection among cognition-related resting-state brain networks.

Results: Compared with healthy subjects, patients with SWD showed impairments on the attention and immediate memory. Seven resting-state brain networks were identified, and patients with SWD showed more numerous granger causality connections in comparison with healthy subjects. Two-sample *t* test results showed that there were significantly increased inflows from the anterior default mode network (aDMN) to sensorimotor network (SMN) and left frontoparietal network (LFPN) to salience network (SN). Correlation analyses showed that the increased inflows from aDMN to SMN were negatively associated with the score of attention, while LFPN to SN were negatively associated with the score of visuospatial/constructional ability.

Conclusions: This study indicates that SWD impairs cognitive performance, and the specific intrinsic brain granger causality connectivity among resting-state networks in SWD patients is affected after long-term shift works.

Keywords: granger causality connection, resting-state functional magnetic resonance imaging, resting-state networks, shift work disorder, cognitive abilities

INTRODUCTION

Shift work disorder (SWD) is a condition defined by excessive sleepiness or insomnia accompanied by total sleep time reduction (1). According to the epidemiology, SWD effects approximately 10–38% of the shift worker population. Nurses make up the largest proportion of shift workers (15–20%) (2). Accumulating evidence suggests that long-term shift work not only affects work efficiency and satisfaction but also leads to physical and mental health problems (3, 4). In hence, given enough time, SWD may lead to more severe disorders, such as cardiovascular diseases, cerebrovascular events, metabolic disorders, gastrointestinal complaints, and multiple forms of cancer (5–7). Meanwhile, SWD also impairs memory, which will directly affect work efficiency or even cause safety consequences not only for the individuals concerned but also for society (8). Therefore, it is essential to evaluate impairments of cognitive abilities for patients with SWD comprehensively and uncover related neuroimaging mechanisms.

In the past decades, functional magnetic resonance imaging (fMRI) develops rapidly, which supplies a noninvasive and convenient method to explore the neuroimaging mechanisms of cognition declines. Numerous fMRI studies have revealed great changes of the brain's intrinsic functional connectivity stemming from cognitive impairments on patients with sleep disorder (9–11). During resting state, brain functional networks including anterior default mode network (aDMN), posterior default mode network (pDMN), sensorimotor network (SMN), left frontoparietal network (LFPN), right frontoparietal network (RFPN), executive control network (ECN), visual network (VN), auditory network (AN), salience network (SN), cerebellum network, and language network are detected by fMRI, which can reflect spontaneous fluctuations and are associated with processing of cognition, emotion, action, and so on (12, 13). Patients with primary insomnia showed increased global functional connectivity strength in the ECN, aDMN, dorsal attention network (DAN), and VN (11). Previous studies have confirmed that DMN connectivity altered during sleep deprivation (14, 15). As another type of sleep disorder, to our best of knowledge, there was only one neuroimaging study, which reported that patients with SWD showed brain perfusion changes in multiple brain areas significantly correlated with insomnia severity (16).

In the study of causal relationships among brain networks, the causality model is suitable to display intranetwork communications, especially information flows among resting state network. The granger causality analysis (GCA) is always applied in studying causalities among brain regions or networks (17). It has been widely used in researches on stroke (18), Alzheimer's disease (19), and so on. However, to our knowledge, there is no study focused on causal relationships of cognition-related brain networks in patients with SWD.

In the current study, we firstly recruited female nurses with SWD as research subjects and evaluated impairments on cognitive abilities. Then, all subjects underwent resting-state fMRI scanning. We extracted resting-state brain networks by independent component analysis (ICA) and applied the multivariate granger model to analyze the intranetwork causality in all subjects. We postulated that there were two different causal connectivity pattern and significant alterations in the seven important cognition-related brain networks in patients with SWD compared with healthy subjects.

MATERIALS AND METHODS

This study was approved by the Beijing Anding Hospital of Ethics Committee. All participants signed informed consents before inclusion in this study.

Participants

Thirty right-handed participants (all females, aged 28.33 ± 2.60 years) met the following inclusion criteria: diagnosed as SWD according to the International Classification of Sleep Disorders (2nd edition) by the American Sleep Disorders Association (20); female nurses working at Beijing Anding Hospital; aged from 18 to 40 years, right-handed; continuous regular night shift for at least 1 year and work for 5 to10 years, at least two shift work per week; with no history of prophylactic or therapeutic medicine in the past 3 months; and with no history of long-term use of analgesics. The exclusion criteria were as follows: pregnant or lactation, history of neurologic or psychiatric disorders, participating in such cognitive experiments within the previous 1 year, any other health disorders or poor physical conditions that may influence participation, any brain structure damage or abnormalites identified by MRI examinations, history of alcohol or any drug dependency, and any MRI contraindications. Another 25 healthy subjects (all females, aged 27.19 ± 2.47 years) were recruited. They followed the inclusion criteria below: relative regularity of sleep in the past 12 months; aged 18 to 40 years, right-handed; and sleeping less than three times per month after 23 o'clock in the latest 1 year and night shift less than three times a month in the previous 1 year.

Cognition and Symptoms Assessment

Prior to MRI scanning, each participant was asked to complete the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) (21) and Pittsburgh Sleep Quality Index (PSQI) to evaluate her attention and sleep quality (22).

RBANS, a cognitive screening test including 12 subtests, was utilized as the measure of global cognition. The RBANS generates five domain-specific index scores applied to evaluate five cognitive abilities: immediate and delayed memory, language, visuospatial/constructional ability, and attention. In spite of delayed memory, a component is based on four subtests, the other four components are based on two subtests. The immediate memory index comprises the story memory and list learning subtests, the visuospatial/constructional index comprises the line orientation and figure copy subtests, and the language index comprises the photo naming and semantic fluency subtests. The attention index comprises the digit span and coding subtests, and the delayed memory index comprises the list recall, story recall, list recognition, and figure recall subtests. The Chinese version of the RBANS translated by

Cheng et al. was adopted in the current study (23). The test undergoes about 30 min. A trained neuropsychologist performed the test according to standardized procedures.

MRI Acquisition

Images were acquired applying a 3.0 Tesla MRI scanner (Siemens, Prisma, Germany) at Anding Hospital, Beijing, China. Prior to scanning, all participants were asked to rest for 30 min before scanning. They were instructed to stay still, think of nothing in particular, keep eyes closed, and not to fall asleep during scanning. Earplugs were worn to attenuate scanner noise. The foam head holders were immobilized to minimize head movements during scanning.

Prior to the functional scanning, we collected high-resolution structural information for anatomical localization by using 3D MRI sequences. The resting-state fMRI data were collected using a single-shot, gradient-recalled echo-planar imaging sequence with the following parameters: repetition time = 2000 ms, echo time = 30 ms, flip angle = 90°, matrix = 64 × 64, field of view = 225 mm × 225 mm, slice thickness = 3.5 mm, gap = 1 mm, 32 interleaved axial slices, and 180 volumes.

In this study, a 490-s resting were scanned first, and then 250-s high-resolution structural scan were employed.

Data Processing

Results included in this manuscript come from preprocessing performed using fMRI Prep 1.5.0 (24), which is based on Nipype 1.2.2 (25).

Anatomical Data Preprocessing

N4BiasFieldCorrection (26) [antsApplyTransforms (ANTs) 2.2.0] was applied for intensity non-uniformity (INU) to correct the T1 images. OASIS30ANTs as a target template was then used to skull-strip the T1 images. Cerebrospinal fluid, white matter, and gray matter were extracted by the command 'fast' (FSL5.0.9). Brain surfaces were reconstructed using recon-all (FreeSurfer 6.0.1). During reconstruction, the brain mask was estimated by the method to re-reconcile ANT-derived and FreeSurfer-derived segmentations (27). Brain structure abnormalites were identified by MRI examinations on two subjects. In hence, the two subjects were ruled out during anatomical data preprocessing.

Functional Data Preprocessing

First, a custom methodology of fMRIPrep was applied to generate a reference volume and its skull-stripped version. The BOLD reference was co-registered to the T1w reference by bbregister (FreeSurfer). Head-motion parameters of BOLD reference were estimated by mcflirt (FSL 5.0.9). After that, slice time was performed using 3dTshift (AFNI). The processed time series were resampled to surfaces (fsaverage5). Framewise displacement (FD), DVARS, and three region-wise global signals were calculated for preprocessed BOLD. Some physiological regressors were also extracted for noise correction (28). Four participants were ruled out for exhibiting head motion of >1.5° rotation maximum translation and 1.5 mm in the process of MRI scanning. Finally, voxel-based resampling was implemented by ANTs. Surface resampling was implemented by mri_vol2surf (FreeSurfer).

RSNs Extraction

ICA was applied to extract the RSNs by GIFT software (University of New Mexico, Albuquerque, NM). The number of independent components in all data was calculated by method of the minimum description length (MDL) technique. Thirty components were estimated. Randlnit and Bootstrap operations were applied to evaluate the independent components. According to research objectives, seven independent components were selected based on the largest spatial correlation comparing with previous resting brain network templates. We selected seven specific networks including ECN, VN, sensory motor network (SMN), LFPN, pDMN, SN, and aDMN.

Network Granger Analysis

All selected components were filtered between 0.01 and 0.1Hz for multivariate granger causal modal to explore the characteristics of networks. Meanwhile the generalized partial directed coherence (GPDC) was selected as the measured parameter (29). The order of GCA was determined by the method of Akaike information criterion. Then, comparisons between groups were analyzed on causal interaction of seven components. One-sample t test in each component was also applied to compute the single network imaging. For comparing the patient group with control group, P value of two sample t test was set as 0.05, which was corrected by false discovery rate (FDR) for multiple comparisons. Finally, BrainNet Viewer was used to display the result onto a 3D brain surface.

Correlation Analysis

As we conducted a comparison between patients with SWD and healthy subjects, we found the patients showed increased inflows from the aDMN to SMN, LFPN to SN. Mean granger causality values between the aDMN and SMN, or LFPN and SN, correlated with the scores of REBANS using Pearson correlation analysis. Statistical analyses were conducted using SPSS 20.0 (SPSS Inc., Chicago, IL, USA), and threshold was set at $P < 0.05$.

RESULTS

Demographic and Clinical Information

Socio-demographic characteristics and PSQI scores of all subjects are displayed in **Table 1**. **Table 1** also showed that the age of patients with SWD distributed between 25 and 30 years old, and all subjects are female, which can eliminate ageing and gender impacts on changes in tolerance to shiftwork.

Compared with healthy subjects, patients with SWD showed declines on attention (t = -9.62, $p < 0.0001$) and immediate memory (t = -5.10, $p < 0.0001$). No significant difference on visuospatial/constructional (t = -0.84, p = 0.40), language (t = -1.89, p = 0.06), and delayed memory (t = -1.20, p = 0.24) was found between the two groups (shown in **Table 2**).

TABLE 1 | The demographic information of patients with SWD and healthy controls.

Items	Patients with SWD (N = 30)	Healthy controls (N = 25)
Gender (male/female)	0/30	0/26
Age (years)	28.33 ± 2.60*	27.19 ± 2.47
Educational level (years)	17.35 ± 1.06#	17.14 ± 0.64
PSQI scores	9.60 ± 3.83	/

**Results from two-sample independent t test of the comparison between two groups, t = 1.68, P = 0.10 (for age); #results from two-sample non-parametric test of the comparison between two groups, z = -0.18, P = 0.86 (for educational level).*

TABLE 2 | The results of RBANS between patients with SWD and healthy controls.

RBANS index score	Patients with SWD (N = 30)	Healthy controls (N = 25)
Immediate memory	90.33 ± 5.88*	99.16 ± 6.96
Visuospatial/Constructional	103.50 ± 4.67	104.56 ± 4.60
Language	96.57 ± 5.76	99.76 ± 6.76
Attention	85.27 ± 7.69*	103.40 ± 5.96
Delayed memory	96.90 ± 4.26	98.36 ± 4.78

*Results from two-sample independent t-test of the comparison between two groups, *p < 0.05. RBANS: Repeatable Battery for the Assessment of Neuropsychological Status.*

ICA Results

Applying ICA in all participants, the SN, aDMN, pDMN, ECN, LFPN, SMN, and VN were extracted. Spatial positional distributions of the seven resting-state networks are shown in **Figure 1** and **Table 3**.

GCA Results

The granger causality of the seven resting-state brain networks in patients with SWD showed different patterns of causal connections compared with healthy subjects. Patients showed more numerous granger causality connections. For the patients, SN and VN were the core networks with more effective connections than others (**Figure 2**, left panels). Two-sample *t* test results showed that there were significantly increased inflows from aDMN to SMN and LFPN to SN (shown in **Figure 2**).

Correlations

To investigate the association between cognitive performances and abnormal casual connectivity, we conducted correlation analysis between mean granger causality values within abnormal casual connectivity and the scores of REBANS. As shown in **Figure 3**, the correlation analysis revealed that the increased inflows from aDMN to SMN were negatively associated with the score of attention (r = -0.49, p = 0.014), while LFPN to SN were negatively associated with the score of visuospatial/constructional ability (r = -0.69, p = 0.0002).

DISCUSSION

In the present study, we attempted to assess cognitive abilities and granger causality connection among cognition-related brain networks on patients with SWD. Our results revealed that patients with SWD showed declines on attention and immediate memory, more numerous granger causality connections among resting-state brain networks than healthy subjects, significant causal relations from aDMN to SMN and LFPN to SN were observed in comparison with healthy subjects. Moreover, the increased inflows were negatively associated with the score of attention and visuospatial/constructional ability.

Shift work disrupts the body's circadian rhythms. Circadian disruption is reliably associated with mood disorders, cognitive function and subjective wellbeing (30). Displaced work hours could change lifestyle factors for shift workers, which may impair cognitive functions (31). In this study, cognitive abilities were assessed using RBANS. Our results revealed that patients with SWD showed declines on attention and immediate memory. This finding is in line with previous studies. One recent research on shift workers showed that driving events were increased following night shifts, and inattention-related events were highest during the

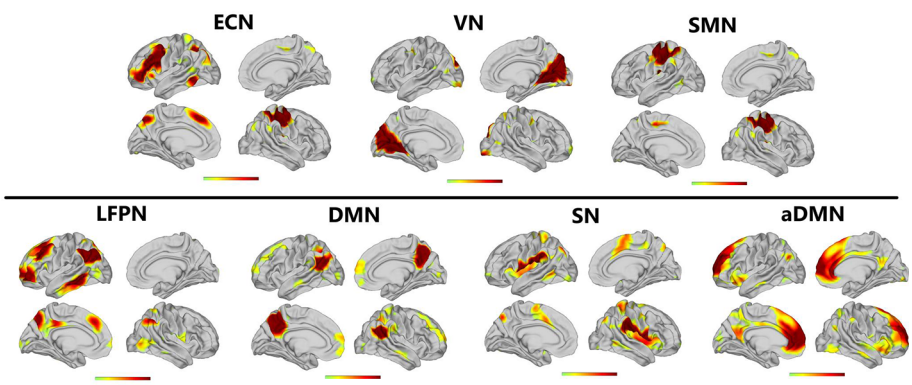

FIGURE 1 | Resting-state networks screened through ICA. The t value (depicted by cold to warm colors) represents the spatial statistical significance of the current networks. L, left; R, right.

TABLE 3 | Spatial positional distributions of brain networks.

Region	Hem	MNI coordinates			t value	Area(mm)
		X	Y	Z		
Executive control network						
Inferior frontal cortex	L	41	28	24	12.16	4199
Intra parietal cortex	L	33	65	42	17.38	2003
Anterior cingulate	L	7	26	47	6.45	550
Temporal cortex	L	54	51	8	6.22	612
Insular	L	28	22	1	4.23	95
Parieto-occipital cortex	L	5	71	45	7.01	432
Prefrontal cortex	R	-46	-25	-28	10.67	2081
Intraparietal cortex	R	-35	-66	-43	10.55	611
Anterior cingulate	R	-7	-40	-40	5.57	460
Insular	R	-30	-22	-1	4.49	156
Visual network						
Posterior cingulate	L	-13	-75	12	20.60	9604
Posterior cingulate	R	20	-66	7	19.68	6824
Sensory motor network						
Precentral cortex	L	-44	-25	61	28.94	4552
Postcental cortex	R	45	-22	60	23.15	4102
Left frontoparietal network						
Dorsolateral prefrontal cortex	L	-41	-28	-24	12.16	5400
Superior parietal cortex	L	-33	-65	-42	17.37	2427
Posterior cingulate	L	-5	-71	-45	7.01	679
Posterior temporal cortex	L	-54	-51	-8	6.22	777
Medial prerontal cortex	L	-7	-26	-47	6.45	776
Superior parietal cortex	R	50	-55	-43	5.61	611
Medial superior temporal cortex	R	46	-77	0	3.14	676
Posterior default mode network						
Superior parietal cortex	L	-52	-54	23	12.89	3162
Posterior cingulate cortex	L	-8	-61	44	16.01	1935
Superior parietal cortex	R	51	-49	25	13.75	1668
Posterior cingulate	R	5	-61	38	16.5326	1464
Superiror temporal cortex	R	60	-16	-15	3.2	771
Salience network						
Insular	L	-62	-30	21	10.83	5207
Middle cingulate	L	-10	7	40	3.29	867
Postcentral cortex	L	-27	-32	68	3.43	977
Superior parietal cortex	L	-32	-73	43	4.53	527
Insular	R	-60	-29	20	10.8	6237
Postcentral cortex	R	24	-46	62	4.4	1312
Middle cingulate	R	7	3	54	3.49	1235
Anterior default mode network						
Anterior cingulate	L	-7	60	18	9.5	9306
Anterior cingulate	R	8	58	13	8.68	9974
Medial superior temporal cortex	R	45	-66	-12	2.2	537

postnight shift commute compared with day and evening shifts, which pointed out that attention was impaired on SWD (32). Another study on sleep and alertness in SWD confirmed that the SWD group had more lapses in psychomotor vigilance tasks compared with the non-SWD group, which also revealed attention impairment in SWD (33). A large cross-sectional sample study showed that memory function tended to decrease with increasing shift-work duration for shift workers (34). An event-related brain potential study demonstrated significant attenuation of mismatch negativity amplitude over frontal regions in patients with SWD, which suggested sensory memory reduction in SWD (35). As circadian misalignment and sleep disruption are detrimental to hippocampus-dependent memory, shift work could impair memory and learning processes (36), which might interpret immediate memory impairment on patients with SWD.

Shift work can result in physiological stress, which have an impact on brain structures and function involved in cognition (37). In our study, resting-state brain functional networks were extracted from patients with SWD. We found that significant causal relations from aDMN to SMN and LFPN to SN were observed in comparison with healthy subjects. The aDMN, SMN, SN, and LFPN are mainly related to cognition and emotion processing. The aDMN is related to self-referential mental activity (38), while SN is the central region for detecting and filtering salient stimuli and modulates cognitive resources including attention for salient stimuli (39, 40). Both networks are associated with regulating emotion. Prior research indicated that SWD can lead to emotional dysregulation, such as negative mood states and frustration (41). Numerous studies revealed altered DMN connectivity during sleep deprivation and even normal variability in hours of sleep the night (14, 42). A system-neuroscience-based meta-analysis confirmed that attention deficit/hyperactivity disorder was associated with disrupted DMN (43). SMN involves in both somatosensory perception and movement generation (44). Circadian rhythmicity could modulate sensorimotor cortices (45), which suggested SWD could influence SMN. Our result also showed that increased inflows from aDMN to SMN were negatively associated with the attention, which may serve as a new potential biomarker for attention decline on SWD.

Patients with insomnia show less functional connectivity variability between the SN and the left executive-control network (ECN) (46). The SN is confirmed to modulate the activation and deactivation of ECN. Meanwhile, LFPN is considered to underpin executive control functions, memory and visual processing, and the ECN and LFPN both monitor executive control functions. A previous study on essential tremor showed that the increased connectivity of LFPN was associated with worse performance on visuospatial ability (47). Another study on obsessive compulsive disorder also revealed hypoconnectivity between frontoparietal control network (FPN) and SN (48). Hence, increased inflows from LFPN to SN in SWD, as well as its negative relationship with visuospatial/constructional ability, may indicate a compromised capability of patients with SWD to interact between the cognition and emotion. These findings interpreted that brain networks were widely hyperarousal even during daytime resting state in patients with SWD and might provide more details to understand the underlying neuromechanism of cognitive impairments and emotional dysregulation, including memory impairment and attention fatigue.

However, there are still several limitations in our current study. First, this is a cross-sectional study, and it is unclear how the cognitive impairments of SWD and disruptions in the granger causality connections change over time. Moreover, cognitive functions tend to decrease with the increases in the duration of exposure to shift work. Longitudinal studies are needed in the future. Secondly, as we only enrolled female participants in our study, it is unclear how granger causality connections and cognitive impairment change on male

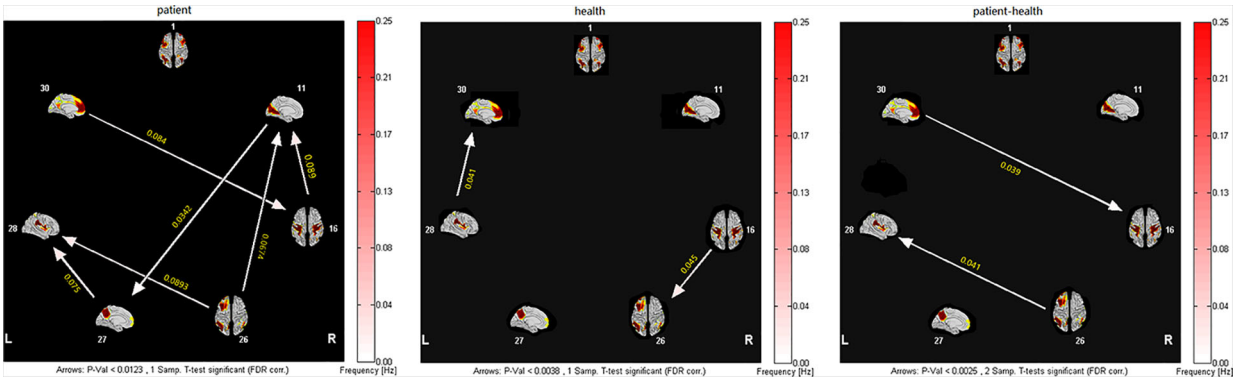

FIGURE 2 | Inter- and intra-group comparisons of patients with SWD and healthy subjects. Panels represent visual descriptions of causal connectivity between 2 networks among the 7 resting-state networks, including 1, ECN; 11, VN; 16, SMN; 26, LFPN; 27, pDMN; 28, SN; 30, aDMN. Arrow directions represent cause and effect. Values on the color bar (corresponding with arrow colors) demonstrate frequency at which causality was found. Left panel: One-sample t test result of inter-group intranetwork causal relationship of MwoA patients. Center panel: One-sample t test result of intergroup intranetwork causal relationship of healthy subjects. Right panel: Two-sample t test result of intra-group intranetwork causal relationship of MwoA patients minus healthy subjects.

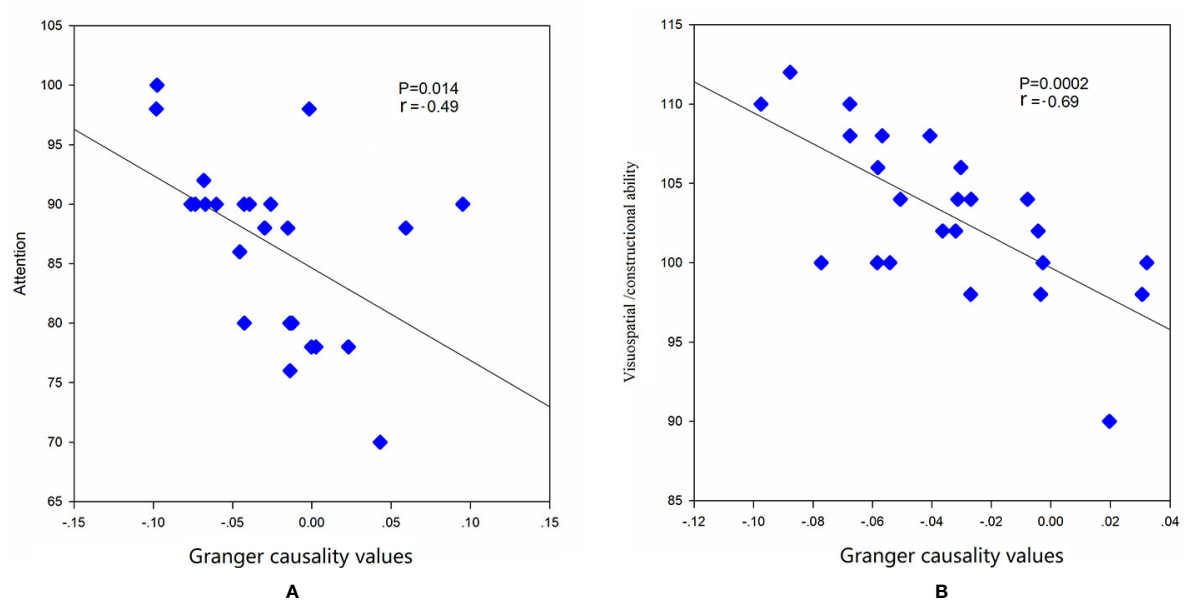

FIGURE 3 | Correlations between mean granger causality values within abnormal casual connectivity and the scores of REBANS. **(A)** The mean granger causality values from aDMN to SMN were negatively associated with the score of attention (r = -0.49, p = 0.014). **(B)** The mean granger causality values from LFPN to SN were negatively associated with the score of visuospatial/constructional ability (r = -0.69, p = 0.0002).

participants. It is necessary to study male patients with SWD in the future.

CONCLUSION

This study indicates that SWD impair brain function and cognitive performance, and the specific intrinsic brain granger causality connectivity among resting-state networks in SWD patients are affected after long-term shift works.

DATA AVAILABILITY STATEMENT

The original data of this study are available from the corresponding authors upon reasonable request.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Beijing Anding Hospital of Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

YN and HJ conceived and designed the study. YN and KL analyzed the data. YN, YZ, PC, DY, and HZ performed the experiment. YN, KL, and HJ drafted the manuscript and gave final approval of the manuscript.

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REVIEW
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Aberrant Awake Spontaneous Brain Activity in Obstructive Sleep Apnea: A Review Focused on Resting-State EEG and Resting-State fMRI

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As one of the most common sleep-related respiratory disorders, obstructive sleep apnea (OSA) is characterized by excessive snoring, repetitive apnea, arousal, sleep fragmentation, and intermittent nocturnal hypoxemia. Focused on the resting-state brain imaging techniques, we reviewed the OSA-related resting-state electroencephalogram and resting-state functional magnetic resonance imaging (rsfMRI) studies. Compared with the healthy control group, patients with OSA presented increased frontal and central δ/θ powers during resting-state wakefulness, and their slow-wave activity showed a positive correlation with apnea–hypopnea index. For rsfMRI, the prefrontal cortex and insula may be the vital regions for OSA and are strongly related to the severity of the disease. Meanwhile, some large-scale brain networks, such as the default-mode network, salience network, and central executive network, play pivotal roles in the pathology of OSA. We then discussed the contribution of resting-state brain imaging as an evaluation approach for disease interventions. Finally, we briefly introduced the effects of OSA-related physiological and mental diseases and discussed some future research directions from the perspective of resting-state brain imaging.

Keywords: obstructive sleep apnea, resting-state, electroencephalography, functional magnetic resonance imaging, brain activity

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INTRODUCTION

As the most common sleep-related breathing disorder, obstructive sleep apnea (OSA) is characterized by excessive snoring, repetitive episodes of apnea, and arousal during various sleep stages. It can lead to severe sleep fragmentation and intermittent nocturnal hypoxemia (1, 2), which may result in excessive daytime sleepiness and increase the incidence of diabetes, hypertension, congestive heart failure, stroke, and cardiovascular disease (3–5). Existing epidemiological studies indicate that OSA is highly prevalent; ∼1 billion people in the 30- to 69-years age group may be affected by OSA, and the prevalence rate exceeds 50% (6, 7). Considering the striking prevalence of this disorder in the general population, the underlying neuro mechanism, however, remains largely unknown.

Over the last two decades, different kinds of techniques, including functional magnetic resonance imaging (fMRI), electroencephalography (EEG), positron emission tomography, magnetoencephalography, and functional near-infrared spectroscopy, have been widely used to investigate the neurophysiological characteristics of OSA. And the EEG-derived polysomnography (PSG) has been considered as the gold standard in the clinical diagnosis of sleep disorders. Among these techniques, EEG and fMRI can collect and analyze data more efficiently, which may have greater application value in the clinical diagnosis of diseases.

With considerable development and wide application of neuroimaging technology, more researchers use resting-state brain imaging to explore the dysfunction of the patient's brain (8). Resting-state brain imaging, especially resting-state EEG (rsEEG) and resting-state fMRI (rsfMRI), was widely concerned for its convenience in operation and straightforward interpretation. During brain scanning, patients just need to lie down or sit quietly for about 5–10 min. The instructions invite the participants to stay relaxed in a state of mind wandering with eyes closed or keep on looking at a cross with eyes open (9). After the recording, the corresponding brain activity and functional connectivity (FC) can be obtained, which may help the clinicians to diagnose and treat the disease. There are some advantages for resting-state brain imaging. First, resting state requires no task-related stimulation, and it has limited requirements on patient's cooperation and interviewer's experience. It also reduced the influence of some irrelevant variables, for example, the familiarity for experiment materials. Second, the results are not dependent on the experimental paradigm, which is conducive to compare among multigroups or cross-center data (8). Third, compared with whole-night PSG, it requires a relatively short time to record. Therefore, the research on resting-state brain imaging is becoming popular, and many big data platforms have been built from the fields of psychology, neuroscience, and clinical radiology (10).

The aim of our review is to summarize the research progress of OSA in the field of resting brain imaging and make suggestions for further research. First, we summarize different kinds of rsEEG and fMRI analytic methods used in the investigation of OSA. Second, we outline the main modality-specific results of OSA research in EEG and fMRI, respectively. Last but not least, current status and future directions of the research of OSA were prospected from the aspects of comorbidities of OSA and some new emerging techniques, such as EEG-fMRI, machine learning, and comorbidity.

RESTING-STATE EEG AND RESTING-STATE FMRI

Neuroimaging techniques have made tremendous progress in the last two decades. Electroencephalography and fMRI are both non-invasive techniques, and more importantly, both have been installed in many research centers and hospitals. At present, rsEEG and rsfMRI are the most widely utilized techniques. Here we focus our review on these two modalities.

Resting-State EEG

As a technique to record the electrophysiological activity of the brain, EEG possesses multiple advantages over other techniques, including high temporal resolution, non-invasiveness, and relatively lower costs. Many investigations employed rsEEG to explore its clinical values for diagnosing or treating OSA. Interestingly, both eyes-closed and eye-open conditions are usually recorded for rsEEG. However, the eyes-closed condition is more widely used, because data in this condition are less contaminated by eye-blinking artifact (11).

As illustrated in **Figures 1A,B**, there are two common analysis methods for rsEEG: power spectrum analysis (PSA) and EEG microstate analysis. Power spectrum analysis is employed to calculate the EEG power of different frequency bands. Electroencephalography signals can be converted from time domain to frequency domain by Fourier transformation, and the rhythms associated with specific neural functions can be extracted and quantified. These rhythms are mainly δ (1–4 Hz), θ (4–8 Hz), α (8–13 Hz), β (13–30 Hz), and γ (>30 Hz) (12). Although some features of PSA are widely used in the researches of OSA, many other features, such as alpha peak frequency, left–right asymmetries, and scale-free properties, are rarely investigated in OSA-related studies.

Electroencephalography microstate analysis investigates brain activity at quasi-stable states of ∼100-ms duration. Based on the clustering of the EEG topography over time, some recurring and stable topographies were identified during resting state (13, 14). Because the duration of the EEG microstate is similar to the duration of a single thought, it was assumed to represent the "atom" of thought (13). Currently, EEG microstate analysis is employed to explore brain changes in mental diseases such as insomnia (15), narcolepsy (16), and schizophrenia (17). However, for our knowledge, there is no study investigating the changes of EEG microstates in patients with OSA.

At present, PSA is the most common analysis method in rsEEG study of OSA. In contrast, many other advanced EEG analysis methods, such as source location (18), brain network analysis (19), and detrended fluctuation analysis (20), are rarely utilized for the studies of OSA. Meanwhile, these advanced analysis methods faced more difficulties in the diagnosis of OSA, because they have higher requirements for hardware, software, and experience. In the future, greater opportunity and challenge may lie in the application of these new methods for the study of OSA. Based on experience and existing research results, we believe that different research methods have different potentials in clinical application, PSA may have higher application value, and the clinical application value of microstate may be low.

Resting-State fMRI

Magnetic resonance imaging was utilized to collect signals of the whole brain with various scanning sequences. Resting-state fMRI is mostly based on the sequence of echoplanar imaging, which reflects the low-frequency spontaneous oscillations (typically 0.01–0.08 Hz) of blood oxygenation level–dependent (BOLD) signals. Brain regions with synchronous BOLD oscillations

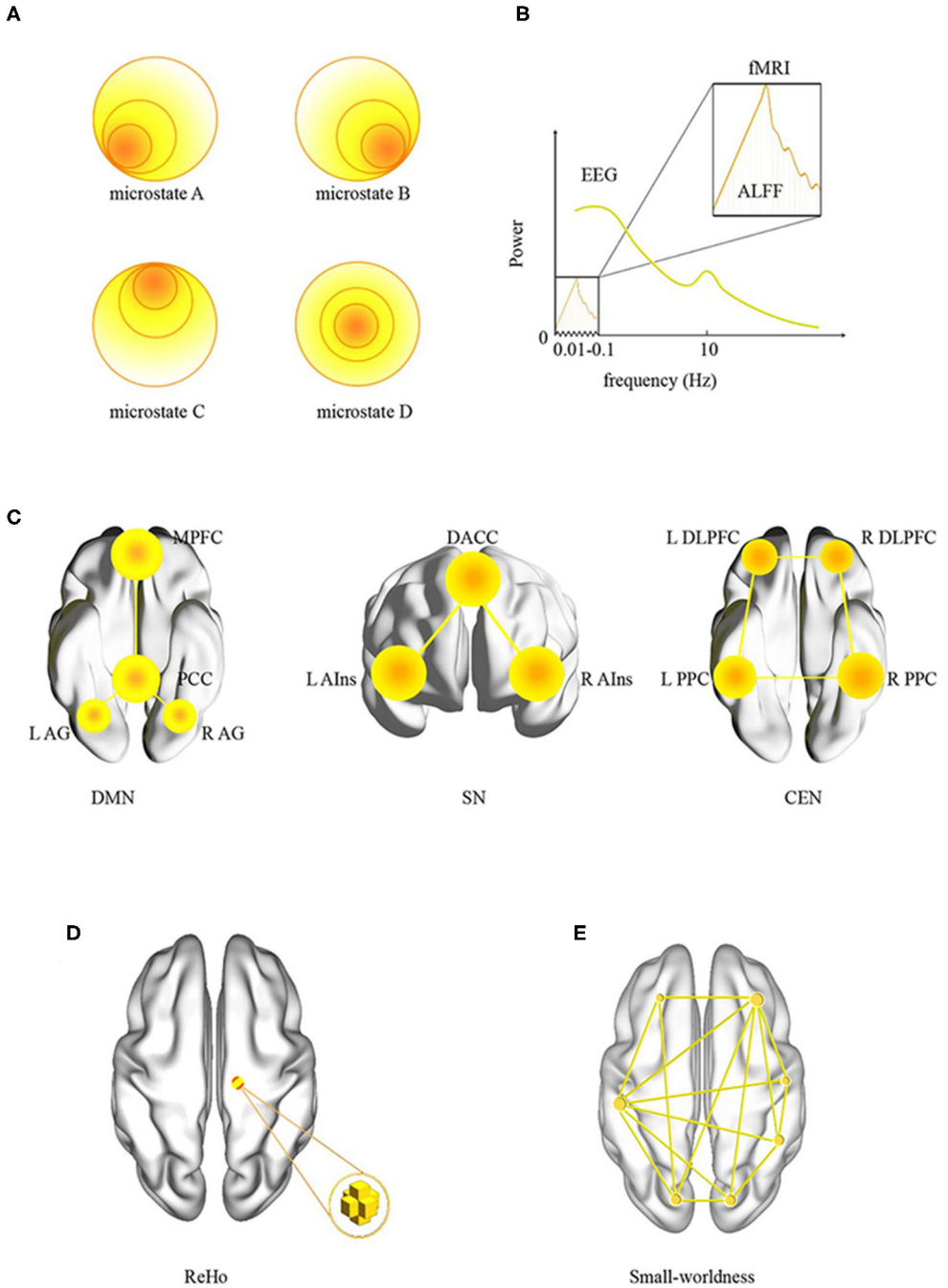

FIGURE 1 | Data analysis methods in resting-state neuroimaging. **(A)** EEG microstates; **(B)** EEG and fMRI power spectra, notice the difference in frequency ranges for each modality; **(C)** large-scale brain networks: DMN, SN, and CEN, and their key regions; **(D)** ReHo; **(E)** small-worldness. ReHo, regional homogeneity; ALFF, amplitude of low-frequency fluctuation; L, left; R, right; DMN, default-mode network; MPFC, medial prefrontal cortex; PCC, posterior cingulate cortex; AG, angular gyrus; SN, salience network; DACC, dorsal anterior cingulate cortex; AIns, anterior insula; CEN, central executive network; DLPFC, dorsolateral prefrontal cortex; PPC, posterior parietal cortex.

constitute a large-scale brain network. For neuroimaging of OSA, some large-scale brain networks receive more attention, such as central executive network (CEN), default-mode network (DMN), and salience network (SN) (21). As illustrated in **Figure 1C**, we listed these three networks and their key regions in the brain.

Most of the previous rsfMRI studies of OSA focused on the local properties (**Figures 1B,D**), that is, the amplitude of

TABLE 1 | Resting-state EEG studies of obstructive sleep apnea.

	References	Number of P/C	AHI of P	Age of P/C	Spectrum change (patients vs. controls)
1	Morisson et al. (26)	21/10	62.9 ± 26.1	44 ± 7/44 ± 6	↑δ, θ activity(frontal) ↑(δ + θ)/(α + β) (frontal, central, parietal, occipital, temporal)
2	Morisson et al. (27)	14/10	62.8 ± 25.8	45 ± 6.4/44.2 ± 6.1	↑δ absolute activity in OSA (frontal) ↑(δ + θ)/(α + β) (frontal, central)
3	Mathieu et al. (28)	(12/13) [young] (13 /14) [old]	46.9 ± 20.3 [young] /42.8 ± 24.7 [old]	38.2 ± 6.4/35.8 ± 8.9 62.2 ± 5.6/60.2 ± 6.4	↑(δ + θ)/(α + β) (frontal, central, parietal, occipital, temporal) in both young and old groups
4	Grenèche et al. (29)	12/8	66.1 ± 11.2	51.2 ± 2.5/49.4 ± 3.4	↑δ, θ, β power
5	Xiromeritis et al. (30)	(28/35/68)/30	10.4 ± 3(mild)/21.3 ± 4.1(moderate)/64.4(severe) ± 18.7(control)	(46.2 ± 11.4/51.3 ± 8.5/49.6 ± 10)/46.7 ± 11.8	↑θ, δ power (occipital, temporal, parietal)
6	Baril et al. (31)	12/12	51.2 ± 23.9	47.9 ± 13.7/44.4 ± 9.5	No change
7	D'Rozario et al. (32)	8/9	49.8 ± 24.7 46.62 ± 7.1	44.6 ± 8.4/27.8 ± 3.7	↑δ power
8	Zeidy Muñoz-Torres et al. (33)	22 [men]/21 [women]/0 [control]	[men]/53.87 ± 7.5 [women]	54.4 ± 2.4 [men]/57.3 ± 2.6 [women]	↓α power ↓β-γ power (frontal, central, moderate OSA, men vs. women) ↓δ power (frontal, central, occipital, severe OSA, men vs. women)

P, patients; C, controls; AHI, apnea–hypopnea index (events/h); PSA, power spectrum analysis; ↑, increased; ↓, decreased; (δ + θ)/(α + β), slowing ratio.

low-frequency fluctuation (ALFF) and regional homogeneity (ReHo). The former measures the spontaneous fluctuations of BOLD signal, whereas the latter focuses on the similarity of the regional signals (22). An interesting topic is a relationship between the power spectrum of EEG and the power spectrum of fMRI. As illustrated in **Figure 1B**, the ALFF focused on a very slow oscillation in fMRI. In contrast, EEG has a wide range of spectrum. They may represent similar neural activity in the frequency range of 0.01 to 0.1 Hz.

Functional connectivity, another commonly used method in rsfMRI, focuses on the statistical correlation between signals in different brain regions. In fact, FC can be divided into two categories: first, seed-based analysis, a model-based region-of-interest (ROI) analysis, requires determining the ROIs in advance based on previous studies or other experiments. Then, a typical process is to calculate the correlation coefficient among the seed regions or with the whole brain (22). Second, independent components analysis (ICA), a model-free analysis, separates BOLD signals into multiple sets of spatiotemporal components. For spatial ICA, the component is spatially independent of each other and constructs a large-scale brain network (21). In recent years, dynamic FC, an extension of static FC analysis, has been developed in rsfMRI. The duration of signal for estimating dynamic FC is a little short, usually <40 s. Dynamic FC may be a new method for the study of OSA (23).

Graph theory analysis is occasionally adopted in OSA studies (24). It provides a relatively simple but powerful quantitative framework to describe whole human brain networks (25). For graph analysis, the nodes can be voxels, ROIs, or even large-scale brain networks. As illustrated in **Figure 1E**, small-worldness is a frequently discussed parameter. With a large clustering coefficient and small average shortest path length, whole brain is statistically imitated as a small-world network.

At present, a growing body of analysis methods has been developed and applied in the study of OSA, which will undoubtedly promote the further exploration of the mechanism of this disease. More importantly, the reliability and validity of these methods are still worth to verify. Besides, based on experience and existing research results, we believe that different fMRI research indicators have different clinical research potentials. For example, graph theory and FC may have lower clinical potentials, whereas ALFF and ReHo may have higher application value.

RESTING-STATE NEUROIMAGING OF OSA

In order to systematically investigate the application of resting brain imaging in OSA, we conducted our search in the Google Scholar, Scopus, and PubMed databases in April 2020 to systematically explore studies using rsEEG and rsfMRI in patients suffering from OSA. The language screening standard of this article is English. The keywords were "(functional magnetic resonance imaging OR fMRI), (electroencephalography OR EEG), (resting state OR rest) and (sleep-related breathing disorders OR sleep apnea OR OSA)." A total of 50 studies were retrieved from the database. Subsequently, we excluded studies that included other technology ($n = 3$), reviews ($n = 2$), and studies that were not related to the main topic of the present review ($n = 21$). The final results included 1,616 participants, with the age range from 4 to 89 years. Among all studies, 8 were about rsEEG (**Table 1**), and 16 were about

TABLE 2 | Resting-state fMRI studies of obstructive sleep apnea.

	References	Number of P/C	AHI of P	Age of P/C	Change (patients vs. controls)
1	Zhang et al. (34)	24/21	54.7 ± 19.9	44.6 ± 7.4/40.6 ± 11.4	FC: ↑PCC(pDMN); ↓MPFC(aDMN), DLPFC(CEN)
2	Santarnecchi et al. (2)	19/19	36.3 ± 13	43.2 ± 8/41 ± 6	ReHo: ↑thalamus, somatosensory, motor ↓right temporal, parietal, frontal
3	Peng et al. (35)	25/25	60.6 ± 18.6	39.4 ± 1.7/39.5 ± 1.6	ReHo: ↑right posterior cerebellum, right cingulate gyrus, lateral lenticular nucleus, putamen, insula ↓nodes of DMN
4	Zhang et al. (36)	24/21	44.6 ± 7.4	44.6 ± 7.4/40.6 ± 11.4	↓FC between right AIns and the nodes of the DMN: MPFC, ACC, SFG, IPL, ITG
5	Li et al. (37)	25/25	60.0 ± 18.6	39.4 ± 1.7/ 39.5 ± 1.6	ALFF: ↓hubs of DMN
6	Taylor et al. (38)	19/17	6 ± 2(no or mild)/28 ± 2(moderate or severe)	58 ± 4/57 ± 4	No change
7	Park et al. (39)	67/75	35.6 ± 23.5	48.0 ± 9.2/47.1 ± 9.3	↓global efficiency, weighting clustering coefficient and nodal centralities
8	Park et al. (40)	69/82	35.6 ± 23.3	48.3 ± 9.2/47.6 ± 9.1	FC changes between insular and many brain regions, PFC, parietal, temporal, cingulate gyrus, basal ganglia, thalamus
9	Li et al. (41)	40/40	59.5 ± 20.9	38.6 ± 8.1/39.3 ± 7.5	↑FC between left IPL and right IPL, and between the MPFC and left and right IPL ↓right hippocampus and the PCC, MPFC, and left MTL
10	Li et al. (42)	36/40	56.5 ± 19.0	39.0 ± 8.1/38.8 ± 11.2	DC ↑lenticular nucleus, the putamen, posterior cerebellar ↓PCC, IPL, left SFG
11	Chen et al. (43)	30/25	62.5 ± 19.2	38.3 ± 8.4/39.5 ± 8.0	↑normalized characteristic path length, local efficiency ↓normalized clustering coefficient, small-worldness, global efficiency
12	Chen et al. (44)	45/45	58.7 ± 20.38	37.56 ± 8.86/37.84 ± 11.38	↑characteristic path length ↓normalized clustering coefficient, small-worldness, global efficiency ↓nodal centralities of DMN,SN,CEN
13	Chen et al. (45)	46/46	58.26 ± 20.37	20–60	↑nodal DC in the ventral medial PFC and the right parahippocampal cortex ↓clustering coefficient, local efficiency, and nodal centralities in the left PCC and DLPFC
14	Huang et al. (46)	29/26	33.67 ± 21.75	39.62 ± 9.95/ 34.46 ± 9.97	↑characteristic path length ↓clustering coefficient, local efficiency, global efficiency
15	Song et al. (47)	70/89	36.0 ± 23.3	48.3 ± 9.2/ 46.4 ± 9.2	↑FC among hippocampus, precuneus, PCC
16	Yu et al. (48)	40/40	60.1 ± 20.45	37.0 ± 8.74	↓FC nodes of DMN (IPL, AG) ↑FC among left DA, anterior lobe of the cerebellum, among left VA, left IFG and left STG, between right VA and left IFG ↓FC between right DA and right PFC

P, patients; C, controls; AHI, apnea–hypopnea index (events/h); ICA, independent component analysis; ReHo, regional homogeneity; FC, functional connectivity; DC, degree centrality; ↑, increased; ↓, decreased; DMN, default-mode network; aDMN, anterior DMN; pDMN, posterior DMN; MPFC, medial prefrontal cortex; PCC, posterior cingulate cortex; CEN, central executive network; DLPFC, dorsolateral prefrontal cortex; AIns, anterior insula; ACC, anterior cingulate cortex; SFG, superior frontal gyrus; IPL, inferior parietal lobe; ITG, inferior temporal gyrus; MTL, medial temporal lobe; ODI, oxygen desaturation index; MOG, Middle occipital gyrus; SMA, Supplementary motor area; MCC, middle cingulate cortex; MTG, medial temporal gyrus.

rsfMRI (**Table 2**). There are more studies based on rsfMRI when compared with rsEEG.

Previous studies mostly focused on the changes in brain activity in OSA patients during sleep. In this article, the resting state was defined as the rest state of wakefulness, and the changes in brain activity in the non-sleeping process were focused on.

Application of rsEEG in OSA

Until now, only seven studies use rsEEG to explore the pathophysiological mechanism of OSA. The features that have been applied in OSA-related resting-state studies could be classified into two categories: the whole band spectrum and the slow-wave activity. According to the spectrum analysis, we found

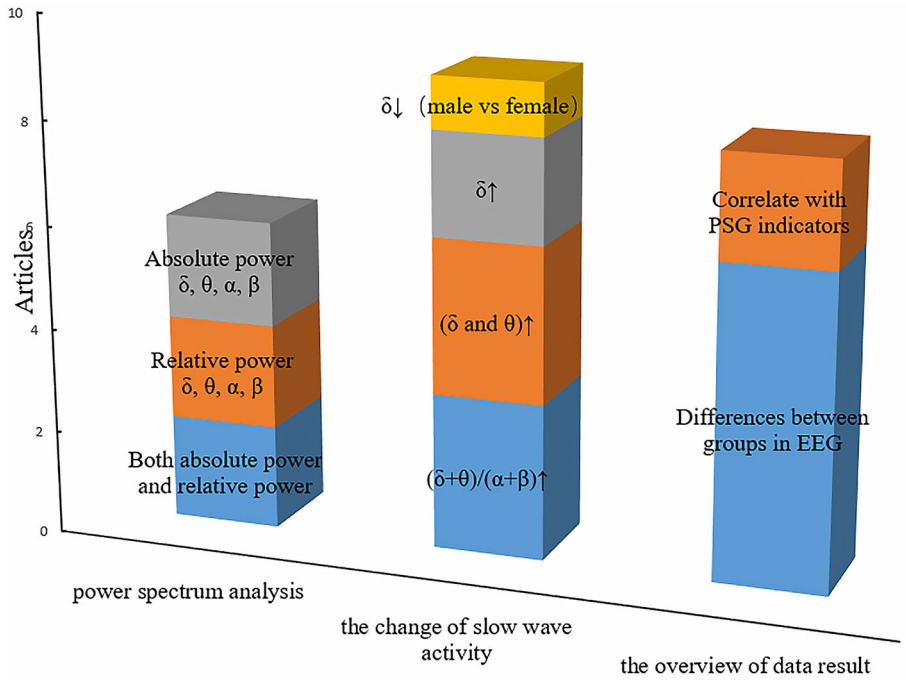

FIGURE 2 | The primary features in resting-state EEG studies of obstructive sleep apnea. The height of the squares of different colors represents the number of articles related to special features.

both absolute power and relative power were equally concerned. In addition, slow-wave activity has shown a consistently increasing trend. For the correlation between rsEEG features and PSG-derived OSA severity indicators, we found there are no positive findings.

Figure 2 summarizes the primary features that were frequently used in OSA studies. The height represents the number of articles for some common features. Both absolute power and relative power were equally concerned, and there were two articles that reported both of them. Another phenomenon is that slow-wave activity is widely reported, with eight articles in total. However, three articles have found an increase in the slowing ratio, three articles have found that both δ and θ are elevated, and only one article has found lower sigma power in men than women in the severe OSA group. In addition, two articles focus on the rise of δ, but not on the changes in θ. Rather than the correlation with PSG indicators, most studies are comparison between OSA patients and health control groups.

For rsEEG, spectrum powers in multiple bands are significantly altered for the OSA patients. Grenèche et al. found an increase in the δ, θ, and β powers, suggesting that maintaining wakefulness requires more cortical activities for OSA patients (29). Six rsEEG studies explored the difference of spectrum power between OSA patients and healthy control group (26, 27, 29–32). Among them, the enhancement of δ activity is a convergent phenomenon. However, very few studies, for example, Baril et al. (31), found no power differences between groups. Five studies found increased low-frequency activity (δ and θ) in the frontal and central regions in patients with OSA compared to healthy controls (26–28, 30, 32). Four studies calculated the slowing ratio (ratio of slow frequencies to fast frequencies) between OSA patients and normal controls. After regressing some confound factors such as weight, age, and education level, these studies found that OSA patients presented a steady increase in the slowing ratio (26–28, 30, 32). In addition, one study found gender differences in brain activity characteristics in OSA. δ, β, and γ powers are lower for men than for women (33). In summary, the increasing power of the low-frequency band, especially the δ rhythm in the frontal and the central areas, is a relatively stable pathological feature for OSA. We speculated that the slow-wave enhancement might be one of the essential criteria for OSA diagnosis and treatment in the future.

To further explore the physiological and psychological mechanisms under abnormal EEG activity in OSA patients, some researchers investigated the relationship between the features of rsEEG and the severity of OSA. Positive correlations between δ and θ relative power and apnea–hypopnea index (AHI) were revealed in patients with moderate to severe OSA (30). Another group reported that the slowing ratio in severe OSA patients was positively correlated with arousal index (ArI) and AHI (28). Not only the slowing ratio, some researchers even found that AHI and α power were positively correlated in OSA patients, and oxygen desaturation index (ODI) was positively correlated with θ and α power. In addition, they found that daytime alertness efforts were related to ArI, and daytime sleepiness was related to ODI (49). Other studies found no significant correlation between OSA severity and any rsEEG rhythm (26, 27, 49, 50). Only

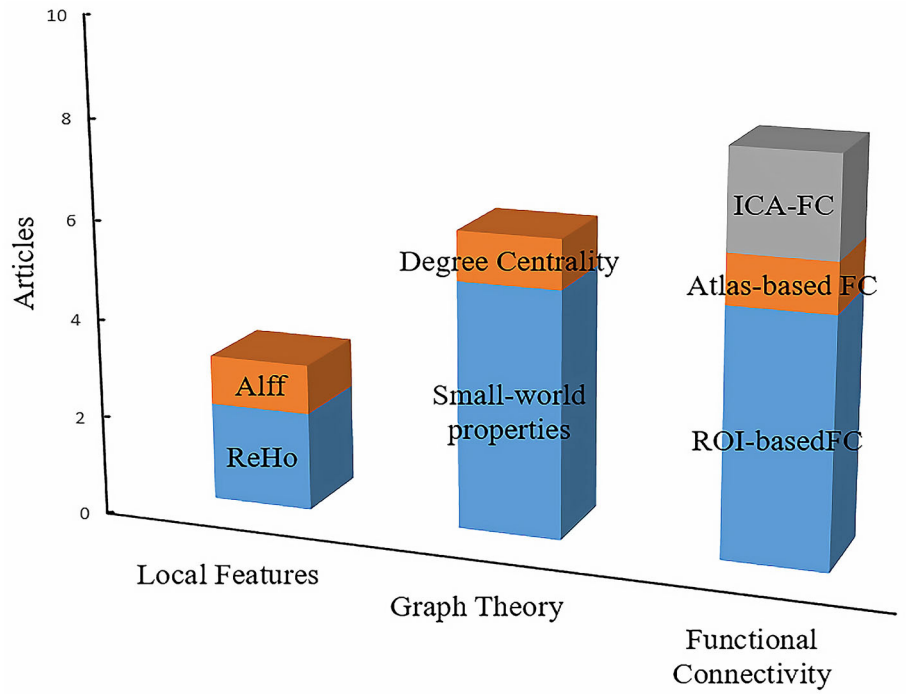

FIGURE 3 | The primary features in resting-state fMRI studies of disorders of consciousness. The height of the squares of different colors represents the number of articles related to the contents of the square. In conclusion, the interpretation of the other column charts is consistent with the **Figure 2**.

two studies found a significant correlation between subjective sleepiness and rsEEG power. And regrettably, the results were inconsistent. In one study, Epworth Sleepiness Scale (ESS) was positively correlated with the δ, θ, and α relative powers in eyes-open condition, and the similar correlation was identified with the δ and α relative powers in eyes-closed condition (32). Another study reported that the ESS was negatively correlated with δ and θ relative powers and positively correlated with α relative power, in eyes-closed and eyes-open conditions separately (30). In addition, no significant differences have been found between genders related to sleep and respiratory factors (33).

In summary, the current results of PSA of rsEEG were relatively consistent. A predominant phenomenon was the increased low-frequency activity, especially in the frontal and central regions. Furthermore, this increased activity was related to daytime sleepiness, which may be caused by long-term hypoxia at night. However, no robust association was found between various indicators of OSA severity and the power of different rsEEG bands. Because of the small number of literature (only eight), a large-scale comparison is impossible in our current review.

Current studies of rsEEG in OSA patients faced some common problems, such as a small number of samples and lack of application of PSG. More importantly, many advanced analyses require more electrodes, so the high-density EEG devices may be necessary. However, the collection of rsEEG data using high-density EEG is a heavy burden for patients, especially patients with severe OSA. It is worth mentioning that PSG cannot completely replace high-density EEG, because the latter has a large number of features and wide coverage of electrodes. For OSA patients, the future study may further consider EEG source imaging based on high-density EEG and may reveal more physiological information with high spatial resolution (18). Besides, the current studies on the resting-state of OSA patients focus on the differences between patients and normal people. Perhaps it may be considered to conduct research based on gender differences, age differences, and different high-risk inducers (such as drinking, obesity, etc.). At the same time, the changes in brain electrical activity of the same patient group in three different states of daytime resting-state, daytime sleepiness state, and night sleep state may also be very interesting.

Application of rsfMRI in OSA

The characteristics that have been applied in OSA-related resting-state studies could be generally classified into three categories: the local features, FC, and the graph theory. **Figure 3** summarizes the primary features that were frequently used in OSA studies. There were three articles that used local features, such as ALFF and ReHo. In addition, we found that FC and ROI-based FC were widely employed to measure the aberrant brain synchronous activity of OSA patients. Graph theory is also increasingly utilized, and it is powerful for investigating the topological properties of the large-scale brain networks (45). In addition, there were three articles that used local features, such as ALFF and ReHo.

For FC analysis, large-scale brain networks, especially the DMN, SN, and CEN, were the focus of the rsfMRI study of OSA (51). The DMN consists of the posterior cingulate cortex (PCC), precuneus, medial prefrontal cortex (MPFC), inferior parietal lobe (IPL), hippocampus, and angular gyrus (AG) and can be further divided into two modules, namely, the anterior DMN and posterior DMN. The main characteristic of the DMN is that it is inhibited during the goal-oriented task, but highly active at rest (21, 22). The CEN mainly includes the dorsolateral prefrontal cortex (DLPFC) and posterior parietal cortex (PPC), and it is thought to be related to cognitive processes such as decision-making and working memory (36). The SN mainly includes the dorsal anterior cingulate and anterior insula (AIns), as well as some subcortical regions, such as the thalamus, striatum, and amygdala. Most of these brain regions are involved in emotions and goal-directed responses. During the execution of specific cognitive tasks, the above three brain networks always cooperate, and the moderation of the SN between the DMN and CEN may be the physiological mechanism of transition from resting state to cognitive processing state (52).

From the perspective of the large-scale brain networks, functional abnormalities of DMN network were often reported in rsfMRI studies of OSA. Six studies have reported abnormal internal connectivity of the DMN, including changes in global and local characteristics of the DMN, FC, and modulation structure (34, 35, 37, 41, 42, 45). Among them, the decrease in FC between the anterior DMN and other nodes of DMN is a relatively consistent result. In contrast, it is still controversial whether the FC within the posterior DMN is abnormal or not (34, 45).

Not only the DMN but also the CEN and the SN networks were reported that they had the abnormal FCs in OSA patients. A dominate change is the FC between the prefrontal lobe and insula. A study led by Zhang found reduced internal FC of DLPFC in OSA patients. In addition, Yu et al. found reduced FC between the right dorsal amygdala (DA) and right PFC (34, 48). Three studies have found that insula, one of the key brain regions of the SN, has impaired or even broken FC with many other brain regions, including prefrontal cortex (PFC), parietal lobe, temporal lobe, and cingulate gyrus (36, 39, 40). For example, a study suggested that the FCs between the right insula and multiple nodes of DMN were decreased. And the similar decrease can be observed in the FCs between the hippocampus and the dorsum medial thalamus, parahippocampal gyrus, and insula, which partly explained the declined working memory ability of OSA patients (36). In addition, the caudate nucleus has an abnormal FC with several nodes in the DMN, especially with the IPL and AG (47). In addition, abnormal FC was revealed in the amygdala subregion, as well as in the left DA and anterior cerebellum (including 4/5 vermis), left ventrolateral amygdala (VA), left inferior frontal gyrus (IFG), and left superior temporal gyrus (STG), and enhanced FC was identified between the right VA and left IFG (48). An analysis of ReHo indicators across the whole brain yielded similar results in the amygdala (2).

Graph theory was utilized occasionally in the rsfMRI studies of OSA. The main work came from the groups of Park, Chen, and others. In Park's study, he and his colleagues found the decrease in global efficiency, weighted clustering coefficient, and node attribute of whole-brain region in OSA patients (40). Compared to healthy controls, OSA patients showed decreased clustering coefficient and local efficiency, increased characteristic path length, and decreased node degree of left PCC and dorsal medial PFC, and the node degree of ventral PFC and right parahippocampal gyrus increased (44, 46). In addition, Chen et al. also found that changes in global and local network properties and changes in the properties of several major nodes reflect the abnormal connection between the three major networks and may be related to cognitive impairment (45). These studies are consistent with the results of previous studies on other indicators. Researchers speculated that chronic nocturnal hypoxemia leads to changes in small-world characteristics of patients' brain networks, which have certain effects on whole-brain function. Although the results of graph theory metrics are sometimes difficult to interpret from behavioral or pathophysiological points of view, the study of patients' small-world network properties and the evolution of their patterns may be one of the indicators to distinguish OSA severity.

In summary, the FC associated with the three large-scale brain networks and their major nodes is significantly altered for OSA patients. The decrease in the strength of connections between different networks may explain the decline in the related functions, whereas the enhancement of FC within some network nodes can be understood as functional compensation to some extent. The evidence from graph theory analysis, such as the increase in characteristic path length and the decrease in global efficiency, is also the evidence for the above opinion. Therefore, reduced FC between large-scale networks and enhanced FC within networks may be utilized as stable biomarkers of OSA disease, which is the distinctive evolution pattern of OSA's influence on brain function. In the future, with the support of more evidence, these FC-based biomarkers may be applied in the screening and diagnosis of diseases. In addition, the current research on OSA mainly focuses on the static local indicators and the calculation of the whole-brain network. Perhaps we can further focus on the dynamic changes of the above biomarkers and the more microscopic differences of these biomarkers in different frequency bands. At the same time, because it is difficult for OSA patients to stay awake for a long time, it is particularly important to explore the transition from wakefulness to daytime sleepiness in OSA patients.

RsfMRI of OSA: Relationship With Severity of Disease

To better understand the relationship between abnormal neuroimaging features and the severity of the disease, investigating the relationship between direct physiology indicators of OSA and quality features of brain imaging is undoubtedly quite important. Physiological indicators from PSG are more representative of OSA severity, especially AHI and ODI. For example, a significant positive correlation between the connections of brain and ODI was found (48). Other researchers, such as Park, demonstrated that the function of the connection of

the left insula and precuneus, IFG, cingulate central operculum, and IFG is positively correlated with AHI, and the connections in the left insula and bilateral sense–motor areas, left middle temporal gyrus, left middle temporal gyrus, left anterior central gyrus, right posterior hippocampus, and right cerebellum were negatively correlated with AHI (40). Zhang et al. found that the FC between the right AIns and MPFC was positively correlated with AHI, negatively correlated with the lowest saturation of blood oxygen (SaO_2), and the internal FC of the right DLPFC was negatively correlated with AHI (36). The right DLPFC internal FC was negatively correlated with AHI (34). A Canadian group suggested that the FC of a small number of voxels in the right AIns is positively correlated with AHI (38), and decreased FCs between the right and left hippocampus and bilateral dorsum medial thalamus were negatively correlated with AHI (47).

Topological properties of brain networks were also correlated with AHI; for example, normalized clustering coefficient was negatively correlated with AHI, and normalized characteristic path length was positively correlated with AHI (43). In a word, according to the physiological indicators of PSG, we can verify the key role of PFC and insula in OSA research, which are closely related to the severity of OSA. Further research on PFC and insula may provide help for a more detailed diagnosis of OSA severity.

CURRENT STATUS AND FUTURE DIRECTIONS

Currently, based on resting-state neuroimaging, the abnormal brain functions of OSA patients were detectable by both the EEG and fMRI techniques. However, clinical research is more challenging to control extraneous variables, and there are still many uncertainties in the study of the brain function of OSA patients. Therefore, many assumptions and research results need to be verified by more rigorous experiments and more exploratory discoveries. In the following part, the future directions of OSA research were discussed with several perspectives, including OSA-related interventions, the contribution of simultaneous EEG-fMRI, machine learning, and comorbidity.

OSA-Related Interventions

Continuous positive airway pressure (CPAP) is the most effective and widely used treatment of OSA. The mechanism of CPAP is elevating collapsed upper airway tissue to prevent airway obstruction (53). It is known that CPAP recovers both AHI and oxygen saturation in OSA patients. However, the evidence from resting-state neuroimaging is very limited. A Korean group found that the slowing ratio in the whole-brain area decreased after treatment (53). Several studies have pointed out that the main finding on the rsEEG is the reduction of θ power (27, 30, 49). For δ power, a study led by Xiromeritis et al. (30) found a significant increase in δ power after treatment, whereas another study found the contrary conclusion (27). We speculate that different phases of CPAP intervention, potential individual variations, and patient tolerance may be the key reasons for the observed different response in resting-state neuroimaging. On the one hand, rsfMRI study that recorded both pre- and post-CPAP treatment is scarce. On the other hand, the current results of rsEEG are always inconsistent. Thus, it is worth discussing whether FCs within any large-scale brain network or any small-world network properties are improved after treatment. Furthermore, differences in treatment duration and frequency should be fully considered for the influence of CPAP treatment.

In conclusion, the various indicators of resting-state brain imaging can be used as a criterion for evaluating the efficacy before and after the intervention and promote a more in-depth understanding of the physiological mechanisms of the disease. Therefore, the application of resting-state neuroimaging is worthy of expectation for the development of clinical interventions.

EEG-fMRI

Obstructive sleep apnea affects patients' daytime behavior by affecting their sleep quality. Therefore, it is undoubtedly necessary to research OSA patients' sleep states, such as sleep quality, sleep structure, and the brain activity during sleep, which requires simultaneous PSG recording during fMRI scanning. Combined with the high spatial resolution of fMRI and the high temporal precision of EEG, simultaneous EEG-fMRI provides evidence from two perspectives, electrophysiology and blood oxygen metabolism, to explore abnormal brain activity in patients with OSA (54).

With the development of experimental facilities, such as MRI, the combination of conventional MR hardware and advanced scan sequences makes the repetition time reduce to 400 ms or even less. Previous studies have discussed the temporal correlation between EEG waveform and BOLD signal (22), suggesting that there are similarities between the characteristics of brain network patterns and spontaneous oscillations of α rhythms. Simultaneous EEG-fMRI may help researchers better understand the physiological mechanisms of OSA disease. And if we can find a certain relationship between the data of the two modalities on OSA, it will be of great reference value for the screening and diagnosis of diseases and even the subsequent intervention. However, because of the small number of relevant experimental studies, clinical application of this technology in diseases other than epilepsy still needs more exploration and attempts.

Machine Learning

Most of the existing studies identify or classify OSA patients by the characteristics of the EEG changes produced by the patients during the sleep stage. In addition, the signal of rsEEG indicates the abnormal electrophysiological activity of patients in different frequency bands (55). Therefore, the slowing ratio calculated by PSA may be used to screen OSA patients before routine medical diagnosis in the future (56). A related short period of rsEEG measurement (usually ∼5 min) can greatly reduce the workload of routine PSG diagnosis and save medical resources. However, its calculation and the norm for health people need to first be constructed.

In the field of diagnosis of clinical diseases, it becomes increasingly popular to achieve high-efficiency auxiliary

diagnosis by the technology of machine learning. If this technology becomes established as part of routine clinical diagnosis, the resting-state neuroimaging may become an immensely valuable tool for clinical practice. Resting-state fMRI can measure patients' brain FC and also can further measure the small-world network parameters and topological properties. Multivoxel pattern analysis (MVPA) using algorithms, such as linear discriminant analysis and support vector machine, can more accurately distinguish the differences in activation patterns of brain regions in different states (57). By combining the MVPA and the resting-state information, patients and normal people can be distinguished (58). Similar work has been done in other diseases to identify some effective biomarkers in patients with Alzheimer disease (AD) (59), bipolar disorder, and major depressive disorder (58). For example, a noteworthy study evaluated four major features by analyzing abnormal resting-state FC and achieved a classification accuracy of 76.1% in distinguishing major depression from healthy controls (60). Therefore, the development of the above technology may play a role in the rapid diagnosis of OSA and the classification of subtypes in the future.

OSA-Related Comorbidity

For the middle-aged and elderly, impairment of daily function due to cognitive impairment may have a significant impact on the quality of life, whether it is caused by OSA or its associated complications. Among them, the decline in the attention, executive function and working memory ability of the elderly is particularly obvious (61). Evidence from fMRI shows that the decreased activation of the anterior cingulate gyrus, dorsal frontal cortex, and PPC in patients with OSA leads to decreased working memory performance (62). The decrease in cingulate gyrus activity may be related to the execution of attention. Therefore, for the study of OSA patients, identifying the specific effect of comorbidities is very important. Besides, the interactions among the comorbidities and OSA are other important question. At present, some literature has reported the association between OSA and AD (63) or related biomarkers (64). Notably, because OSA patients tend to have multiple complications, it is difficult to fully control each of these complications in clinical practice, making it difficult to distinguish the neural substrate of cognitive impairment. Despite the difficulties, some studies also have tried to explore this aspect. After specifically recruiting OSA patients without complications, a study found that there was no significant difference in cognitive performance between the patient group and the healthy control group (65). Although this study suggests that OSA patients' cognitive impairment may not originate from OSA itself, it is difficult to control the influence of intelligence, education, and other confound factors. And some of the comorbidities may be caused by damage to brain areas caused by chronic hypoxemia and poor sleep quality. Therefore, the traceability of cognitive impairment may also be closely related to the severity of OSA, and the complex interactions between them are difficult to distinguish and quantify. Thus, the above conclusions require a lot of research and large sample studies.

CONCLUSIONS

This article summarizes the literature on rsEEG and rsfMRI studies of OSA so far and provides some suggestions for the better intervention of OSA deterioration and alleviating its impairment on cognitive functions. In summary, evidence from the rsEEG focused on the increased power of δ and θ in the frontal and central regions of the patient, whereas evidence from rsfMRI mainly found functional abnormalities within and between the three large-scale brain networks, that is, the DMN, the SN, and the CEN.

In terms of intervention therapy, we need to pay more attention to the following aspects: reducing the diagnosis cost of patients and improving the unhealthy lifestyles of patients. In terms of the physiological mechanism of diseases, the existing rsEEG research focuses more on the specific rhythms and the abnormity of power, but lacks the research on the connections in various brain regions, so methods such as functional connection and EEG source localization are required. Meanwhile, most of rsfMRI studies pay attention to abnormal activity of large-scale brain networks, and less research has related these abnormalities with the severity of the disease. In addition, the existing studies have not combined the evidence of both EEG and fMRI to explain the pathology of OSA. It is expected that this review will promote our understanding of the resting-state characteristics of neuroimaging studies in OSA.

AUTHOR CONTRIBUTIONS

XL, WZ, and XW contributed conception and design of the study. YW performed the statistical analysis and wrote the first draft of the manuscript. XC wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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Identification of Veterans With PTSD Based on EEG Features Collected During Sleep

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Background: Previously, we identified sleep-electroencephalography (EEG) spectral power and synchrony features that differed significantly at a population-average level between subjects with and without posttraumatic stress disorder (PTSD). Here, we aimed to examine the extent to which a combination of such features could objectively identify individual subjects with PTSD.

Methods: We analyzed EEG data recorded from 78 combat-exposed Veteran men with (n = 31) and without (n = 47) PTSD during two consecutive nights of sleep. To obviate the need for manual assessment of sleep staging and facilitate extraction of features from the EEG data, for each subject, we computed 780 stage-independent, whole-night features from the 10 most commonly used EEG channels. We performed feature selection and trained a logistic regression model using a *training* set consisting of the first 47 consecutive subjects (18 with PTSD) of the study. Then, we evaluated the model on a *testing* set consisting of the remaining 31 subjects (13 with PTSD).

Results: Feature selection yielded three uncorrelated features that were consistent across the two consecutive nights and discriminative of PTSD. One feature was from the spectral power in the delta band (2–4 Hz) and the other two were from phase synchronies in the alpha (10–12 Hz) and gamma (32–40 Hz) bands. When we combined these features into a logistic regression model to predict the subjects in the *testing* set, the trained model yielded areas under the receiver operating characteristic curve of at least 0.80. Importantly, the model yielded a *testing*-set sensitivity of 0.85 and a positive predictive value (PPV) of 0.31.

Conclusions: We identified robust stage-independent, whole-night features from EEG signals and combined them into a logistic regression model to discriminate subjects with and without PTSD. On the *testing* set, the model yielded a high sensitivity and a PPV that was twice the prevalence rate of PTSD in the U.S. Veteran population. We conclude that, using EEG signals collected during sleep, such a model can potentially serve as a means to objectively identify U.S. Veteran men with PTSD.

Keywords: electroencephalography, sleep-stage independent, classification, sleep, PTSD, spectral power, synchrony

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INTRODUCTION

Sleep disturbances are a hallmark of posttraumatic stress disorder (PTSD) (1). For this reason, previous studies have analyzed electroencephalography (EEG) data from overnight sleep-polysomnography (PSG) recordings to identify differences in sleep patterns between groups of subjects with and without PTSD (2–5). Motivated similarly, but with the intent to find differences that are reproducible, we recently identified EEG spectral powers that discriminate combat-exposed Veterans with and without PTSD at the group-average level (6). Specifically, we split the sleep-study data into a set for initial discovery and a set for testing reproducibility of our findings. In that study, we found that the features that showed group-level differences were consistent across two consecutive nights in the initial discovery set and, importantly, that these findings were largely reproducible on the held-out test set. More recently, analyzing the data from the same study using a similar procedure, we found that the synchrony of EEG signals between channel pairs [the average phase difference between two time-series signals over a given time interval (7)] could also significantly discriminate the two groups (8). Another recent study by Modarres et al. (9)—the only publication to date that investigated synchrony between EEG channels in PTSD subjects during sleep—also reported group-level differences, although they did not assess the reproducibility of their results across multiple nights or in an independent dataset. Together, these findings suggest that EEG spectral power and synchrony features can distinguish differences between groups of subjects with and without PTSD (2–5).

The natural next step is to investigate whether these and similar features can be used to diagnose PTSD at the individual level. Current methods of PTSD diagnosis are subjective, relying on a clinician's judgement and a patient's self-report in questionnaires, such as the clinician-administered PTSD scale (CAPS) (10) and the insomnia severity index (ISI) (11). In contrast, an objective means to identify subjects with PTSD would aid clinicians in adjudicating true cases with increased specificity and enable them to track the responses of patients to treatment, while providing the opportunity to shed light on the neurophysiological mechanisms of PTSD (12).

Here, encouraged by the promising findings in the above-mentioned group-difference studies (6, 8), we aimed to assess whether a multivariate classifier, developed using EEG spectral power and synchrony features, could objectively identify individual subjects with PTSD. To this end, we analyzed EEG data recorded from 78 combat-exposed Veteran men with ($n = 31$) and without ($n = 47$) PTSD during two consecutive nights of sleep. Following our recent work, we developed a multivariate classifier using a *training* set, which consisted of the first 47 consecutive subjects (18 with PTSD) of the study, and evaluated the classifier on the *test* set, which consisted of the remaining 31 subjects (13 with PTSD), in order to assess the reproducibility of our findings. In this procedure, we used stage-independent, whole-night features computed on data from the 10 most commonly used EEG channels to facilitate comparison of results across laboratories, because most PSG studies record EEG data from 10 or fewer channels.

METHODS

We recruited combat-exposed Veterans who provided written informed consent in accordance with the protocol approved by the University of Pittsburgh Institutional Review Board (Pittsburgh, PA) and the U.S. Army Medical Research and Development Command Human Research Protection Office (Ft. Detrick, MD). We excluded subjects with any of the following conditions from the study: a current diagnosis of untreated severe depression, psychotic or bipolar disorder, substance or alcohol abuse in the previous 3 months, or sleep disorders other than insomnia or nightmares. It should be noted that we did not exclude subjects with a prior history of alcohol consumption, because doing so would have greatly reduced the sample size and, importantly, the generalizability of our results to Service member populations, in which alcohol consumption is common. All subjects were free of any sleep-related medication for at least 2 weeks prior to enrollment in the study. Before their arrival at the laboratory, we assessed subjects' habitual sleep patterns for 10 consecutive days using a sleep diary (**Table 1**). During this time, we also instructed them to take no more than two cups of coffee per day (or the equivalent caffeine dose) and limit their alcohol intake to two drinks per day over a 2-week period before the study. We also assessed the presence and severity of PTSD via the CAPS (10), the presence of alcohol use disorder in the past month, sleep quality via the Pittsburgh sleep quality index (13) and the ISI (11), and self-reported measures of depression via a patient health questionnaire (14).

Subjects spent two consecutive nights and days in the University of Pittsburgh Medical Center's sleep laboratory. On Night 1, they arrived at 20:00 and were fitted with a PSG system, which consisted of a 64-channel high density-electroencephalography (hd-EEG) montage [HydroCel Geodesic Sensor Net (without sponge inserts); Electrical Geodesics Inc., Eugene, OR] and bipolar channels for submentalis electromyogram signals. Subjects were allowed to sleep undisturbed from 23:00 until 07:00, while we recorded their EEG data. On the morning of the next day, we removed the PSG system and asked the subjects to perform multiple tests to assess daytime alertness and cognitive functions. At 21:00, we refitted the subjects with the PSG system and repeated the same procedures on Night 2 and the following day until their discharge at 20:00.

Among the 85 subjects who completed the study, 37 (six women) met the diagnostic criteria for PTSD and 48 did not (one woman). We excluded all seven women from our analysis to avoid confounding effects due to sex differences (15). The remaining 78 men (31 with PTSD), who ranged from 24 to 51 years of age, comprised our study population (**Table 1**). We split this sample into a *training* set comprising the first 47 consecutive subjects of the study (18 with PTSD) for model development and a *test* set comprising the remaining 31 subjects (13 with PTSD) for assessing model performance.

TABLE 1 | Clinical characteristics and sleep-diary variables for the 78 combat-exposed Veteran men in our study.

Variable	PTSD (n = 31) Mean (SD)	Non-PTSD (n = 47) Mean (SD)	Group comparison *p*-value[a]
Age (y)	31.3 (4.7)	32.8 (6.2)	0.358
Sleep diary[b]			
Time in bed (min)	453.0 (100.6)	465.0 (55.3)	0.580
Total sleep time (min)	414.3 (77.0)	444.1 (52.5)	**0.035**
Sleep efficiency (%)	92.8 (9.5)	95.6 (3.4)	**0.004**
Sleep latency (min)	27.8 (17.1)	10.0 (5.9)	**<0.001**
CAPS	51.4 (16.8)	8.6 (7.9)	**<0.001**
Hyperarousal	19.0 (7.1)	3.3 (4.0)	**<0.001**
Intrusion	10.7 (5.8)	0.6 (1.8)	**<0.001**
Avoidance	16.9 (8.8)	1.7 (3.5)	**<0.001**
Current[c] AUD (n)	2	0	–
Past[d] AUD (n)	17	10	–
PSQI	8.9 (2.8)	4.1 (2.4)	**<0.001**
ISI	14.2 (4.8)	3.8 (4.2)	**<0.001**
PHQ-9	5.8 (2.6)	1.4 (2.5)	**<0.001**

[a]Wilcoxon rank-sum test, bold values indicate p < 0.05; [b]PTSD, n = 30; [c]Present in the past month; [d]Absent in the past month. AUD, alcohol use disorder; CAPS, Clinician-Administered PTSD Scale; ISI, Insomnia Severity Index; PHQ-9, Patient Health Questionnaire-9; PSQI, Pittsburgh Sleep Quality Index; SD, standard deviation.

TABLE 2 | Sleep architecture measures for subjects with and without PTSD during two consecutive nights of sleep at the University of Pittsburgh sleep laboratory.

Measure	Night 1		Night 2	
	PTSD (*n* = 31)	Non-PTSD (*n* = 47)	PTSD (*n* = 31)	Non-PTSD (*n* = 47)
Total sleep time (min)	406.4 (36.1)	411.4 (34.3)	416.5 (27.1)	428.6 (35.3)
Sleep efficiency (%)	84.6 (27.8)	85.7 (7.9)	86.7 (5.6)	89.5 (7.4)
Stage N1 (%)	11.4 (4.8)	10.9 (5.6)	9.7 (3.8)	8.6 (4.6)
Stage N2 (%)	57.6 (7.4)	55.7 (7.2)	55.3 (7.2)	53.3 (6.5)
Stage N3 (%)	8.8 (6.8)	12.6 (7.4)	10.9 (5.9)	13.8 (7.2)
REM (%)	22.3 (5.7)	20.8 (5.3)	24.2 (5.6)	24.3 (5.9)

Values within parentheses denote standard deviations.
REM, rapid eye movement sleep; N1, N2, and N3, non-REM stages of sleep.
None of the Wilcoxon rank-sum tests were significant at the p < 0.05 level, when we compared each of the measures between PTSD and non-PTSD, for each night.

EEG Preprocessing and Feature Computation

We recorded hd-EEG data referenced to the linked mastoids at a sampling rate of 250 Hz. We visually scored sleep stages in 30-s epochs according to the criteria of the American Academy of Sleep Medicine (16). **Table 2** shows the sleep architecture parameters for the study population.

We applied a band-pass filter to preserve the EEG signals within the bandwidth of interest (0.5–50.0 Hz), while suppressing noise in frequency bands outside this range. Next, to minimize the impact of muscle movement in the EEG data, we segmented the data in each EEG channel into 5-s epochs and rejected transient, high-frequency activity whenever the power in the 26.0–50.0 Hz band of each epoch exceeded its moving median value over a 3-min window by a factor of four, as previously described (17, 18). Further, to eliminate artifacts due to body and head movement as well as poor electrode contact in each EEG channel, we removed the 5-s epochs for which the power in the 4.0–50.0 Hz band exceeded the whole-night median by a factor of six (6).

We computed three types of frequency domain EEG features—two to capture the mean and the coefficient of variation (the ratio between the standard deviation and the mean) of the log EEG power spectrum for each channel, and a third to capture the phase synchrony between pairs of EEG channels (with 1 denoting perfect synchrony and 0 denoting no synchrony) (7). We computed each of these features over the following 12 frequency bands spanning 0.5 to 40 Hz: 0.5–1 Hz (slow oscillations); 1–2 Hz [low delta (Lδ)]; 2–4 Hz [high delta (Hδ)]; 4–6 Hz [low theta (Lθ)]; 6–8 Hz [high theta (Hθ)]; 8–10 Hz [low alpha (Lα)]; 10–12 Hz [high alpha (Hα)]; 12–14 Hz [low sigma (Lσ)]; 14–16 Hz [high sigma (Hσ)]; 16–24 Hz [low beta (Lβ)]; 24–32 Hz [high beta (Hβ)]; and 32–40 Hz [low gamma (Lγ)].

Feature Computation

Our objective was to develop a multivariate classifier that discriminates subjects with and without PTSD. As such, we aimed to reduce false associations due to temporal variations in the aforementioned features over the 8-h sleep period by studying their values averaged across the entire night disregarding sleep-stage specific information. Consequently, the averaged feature values contained the most information from the longest sleep stage, namely, the non-rapid eye movement (NREM) stage, which constituted more than 53% of the 8-h sleep period in both nights (**Table 2**). Furthermore, to increase the generalizability of our results to other sleep studies, we restricted our analyses to the 10 most commonly used EEG channels that cover the whole brain (**Figure 1**). Thus, for each subject, we computed a total of 780 whole-night features independent of sleep stage: 120 log powers (LP; 10 channels × 12 frequency bands), 120 coefficients of variation (LCV), and 540 phase synchronies [the weighted phase lag index (W); 10 × 9/2 channel pairs × 12 frequency bands]. Henceforth, we used the following naming convention for the features: <feature-type>-<channel or channel pair>-<frequency band>. For example, we denoted the log power in the C3 channel in the low-delta band as LP-C3-Lδ and the phase synchrony between the C3 and F3 channels in the high-beta band as W-C3-F3-Hβ.

Feature Processing

We performed the following operations to process the features for use in a multivariate classifier. First, to avoid problems due to heteroscedasticity during classifier development, we log-transformed the synchrony features to scale them similarly to the log features LP and LCV. Second, we used the concordance correlation coefficient (19) to assess the consistency of feature values across the two consecutive nights in the *training* set, and

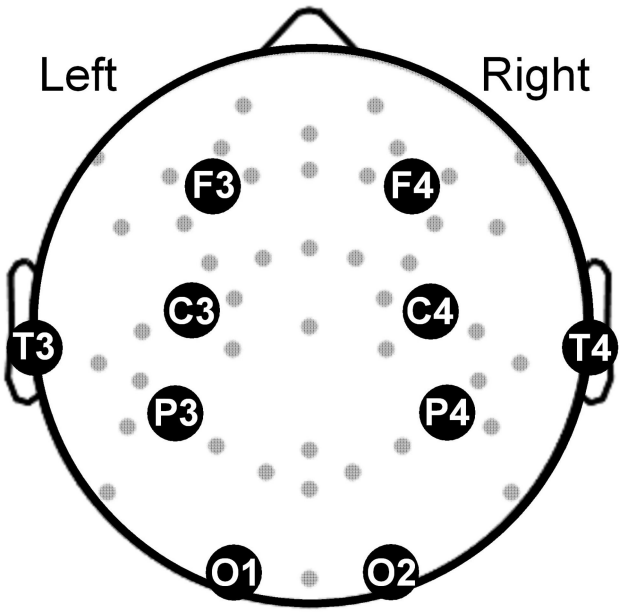

FIGURE 1 | Topographical map showing the electroencephalography channels (gray dots) covering the brain. Large filled circles along with abbreviated names mark the locations of the 10 channels used in our analysis. F3 and F4: frontal channels; C3 and C4: central channels; T3 and T4: temporal channels, P3 and P4: parietal channels; O1 and O2: occipital channels.

retained features with a correlation that exceeded 0.7 (**Figure 2**, step 2). Third, to preclude confounding effects due to age (20), we first computed Pearson's correlation between each feature and age for subjects without PTSD in the *training* set. [It should be noted that we used only subjects without PTSD because determining correlation coefficients based on subjects with PTSD could potentially remove disorder-related changes (21)]. Then, for each feature that was significantly correlated with age ($p < 0.05$), we estimated the parameters of a linear regression model relating age to that feature and used them to statistically remove the effect of age in the entire population (22) (i.e., all subjects in the *training* and *test* sets; **Figure 2**, step 3).

Univariate Feature Selection and Clustering

Following the processing steps, we performed a univariate analysis to select features discriminative of PTSD in the *training* set using the area under the receiver operating characteristic (ROC) curve (AUC) as the metric. For each feature, we computed the AUC and the corresponding lower and upper bounds of the 95% confidence interval (CI) and selected features for which the lower bound of the CI exceeded 0.5 on each of the two nights to guard against chance associations (**Figure 2**, step 4). For all subsequent steps of the analysis workflow (**Figure 2**, steps 5–8), we concatenated the feature values from each of the two nights of the *training* data into a single vector for each feature. To identify and remove correlated features, we clustered the feature vectors using a dendrogram with the distance correlation (23) as a metric. Whereas, a Pearson's correlation of 0 between two features only indicates uncorrelatedness in a linear sense, a distance correlation of 0 between two features indicates that they are independent. Also, unlike Pearson's correlation, which can take values between −1 and +1, the distance correlation ranges between 0 (independent) and 1 (perfect linear correlation). We performed the clustering step because training a classifier using correlated features can result in overfitting, due to the increased number of redundant parameters in the model, and reduced performance on the *test* set. To avoid these problems, we grouped features with a distance correlation exceeding 0.7 into a cluster (**Figure 2**, step 5).

Multivariate Feature Selection and Classifier Development

For each cluster consisting of two or more features, we chose the feature with the highest AUC of the concatenated feature vector, as the representative feature for that cluster. To these (cluster-derived) features, we added the remaining (independent) features, which did not group into any of the clusters, for classifier development (**Figure 2**, step 6). Subsequently, to further reduce the chance of overfitting, we performed recursive feature elimination (24) via six-fold cross validation with a logistic regression model to obtain the smallest set of features for which the regression coefficients were non-zero (**Figure 2**, step 7).

Model Development and Evaluation

We developed the logistic regression model using the smallest feature set on the combined *training* data from both nights, and assessed model performance on data from each of the two nights of the *test* set. We evaluated model performance by using the AUC score and by computing sensitivity, specificity, and positive predictive value (PPV) of the model predictions for different threshold values. Unlike sensitivity and specificity, the PPV depends on the prevalence of PTSD in a given population. In the present study population, the prevalence of PTSD [39% (31 of 78 subjects)] was higher than the estimated value (∼15%) in the overall population of combat-exposed Veteran men (25–27). Therefore, to avoid overestimating the PPV, we re-wrote the standard formula so that the PPV (28), henceforth termed as "adjusted PPV" is an explicit function of the prevalence of PTSD in the overall population (see Note A in the **Supplementary materials**).

We performed all of the aforementioned analyses via custom scripts written in MATLAB (The MathWorks Inc., Natick, MA), as well as in Python version 3.5.2 using the numpy, scipy, sklearn, and pandas libraries.

RESULTS

Feature Selection

Of the 780 features computed for each subject, 454 were concordant (i.e., the concordance correlation coefficient exceeded 0.7) across the two consecutive nights of recordings on the *training* set (**Figure 2**). Among these, 86 features were significantly correlated with age in subjects without PTSD in the *training* set ($n = 29$), which were then corrected to remove

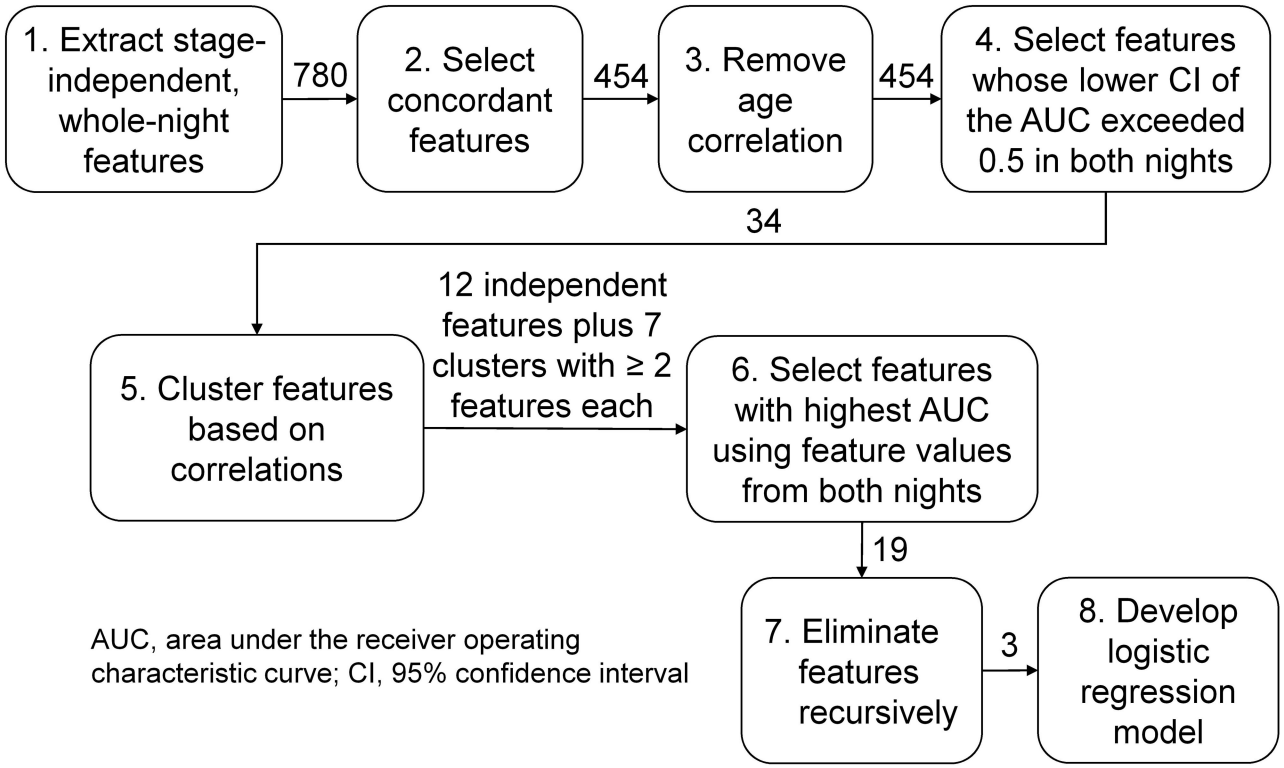

FIGURE 2 | Analysis workflow for feature selection and classifier development. We analyzed three types of sleep-stage independent features, (1) log powers, (2) their coefficients of variation, and (3) the phase synchrony between pairs of electroencephalography channels, averaged across the entire night, in twelve frequency bands of interest. We started with 780 features (120 type 1, 120 type 2, and 540 type 3) and ended up with three features, one of type 1 and two of type 3.

age effects from our study population. We then computed the univariate AUCs and the corresponding 95% CIs for each of the 454 features, and selected the 34 for which the lower bound of the CI exceeded 0.5 in each of the two nights of the *training* set. As noted above in the Methods Section, for all subsequent processing steps (**Figure 2**, steps 5–8), we concatenated the feature values from the two nights of the *training* data to form a single vector for each feature. Distance correlation-based clustering revealed seven clusters with two or more features each (with distance correlation > 0.7) plus 12 independent features that did not cluster with any other feature (**Figure 3**). For each of the seven clusters, we then selected the feature with the highest AUC as the representative of that cluster, forming a total of 19 ($7 + 12$) independent features for further analysis.

Model Development and Evaluation

Using the concatenated vectors for the 19 features, we performed recursive feature elimination using the logistic regression model, which resulted in a final model consisting of three features with non-zero model coefficients (**Figure 2**, step 8). One of the features was LP-C3-Hδ, whereas the other two were phase synchronies in the high-alpha and low-gamma bands. We provide their values and group differences in **Supplementary Tables S2–S4**. The final model, combining these three features, yielded a *training*-set AUC of 0.83 on the combined data from the two nights and *test*-set AUCs of 0.84 for Night 1 and 0.80 for Night 2 (**Table 3**). These values were considerably larger than the univariate *test*-set AUCs of any of the three features, which ranged from 0.55 to 0.74 across the two nights (**Table 4**), indicating superior performance for the multivariate classifier.

We used the ROC curve of the regression model outcome on the *training* set to search for two thresholds that correspond to sensitivity values above 0.80 and 0.90. This search yielded thresholds of 0.37 and 0.26, which corresponded to *training*-set sensitivities of 0.81 and 0.92, respectively. At the threshold of 0.37, the model yielded *test*-set sensitivities that were much lower than the *training*-set sensitivity (0.62 on Night 1 and 0.54 on Night 2 for the *test* set vs. 0.81 for the *training* set; **Table 3**). In contrast, at the 0.26 threshold, the *test*-set sensitivity was 0.85 on each of the two nights (**Table 3**), while the specificities were 18% higher than those of the *training* set (0.67 on each of the two nights for the *test* set vs. 0.57 for the *training* set; **Table 3**). Importantly, the adjusted PPV was 0.31 for the *test* set, which was twice the PTSD prevalence value of 0.15 in combat-exposed Veteran men (**Table 3**).

At thresholds corresponding to a *training*-set sensitivity of 0.92 for each of the three individual features used in the model, the univariate *test*-set sensitivities ranged from 0.77 to 1.00 over the two consecutive nights (**Table 4**), which were comparable to the sensitivity of the regression model (0.85; **Table 3**). However,

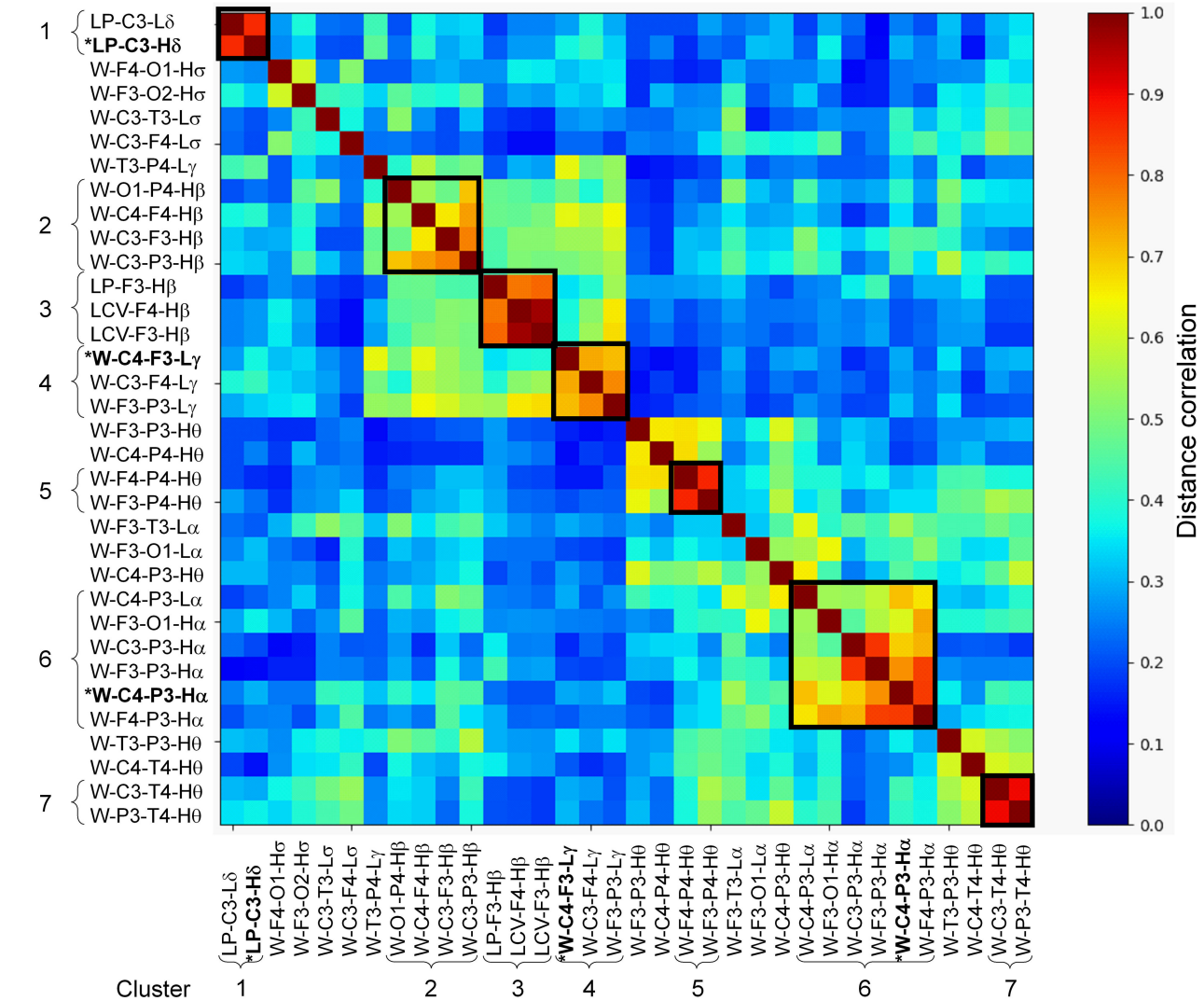

FIGURE 3 | Distance correlation between the 34 stage-independent, whole-night features extracted from 10 electroencephalography channels across both nights of the *training* set (those obtained after step 4 in **Figure 2**). Dendrogram clustering revealed seven clusters with correlation values exceeding 0.7 (dark squares in the image, where the curly brackets identify the features within a cluster). Features marked with asterisk and highlighted in bold-face text (one each in clusters 1, 4, and 6) indicate the three features selected via recursive feature elimination. The 12 independent features obtained after step 5 in **Figure 2** are located between the seven clusters.

the *test*-set specificities (range: 0.06–0.33; **Table 4**) and adjusted PPVs (range: 0.14–0.26; **Table 4**) were much smaller than the corresponding values for the regression model (specificity = 0.67, adjusted PPV = 0.31; **Table 3**). These results further underscore the advantage of combining the three features to identify individuals with PTSD.

DISCUSSION

We found that a logistic regression model, using three stage-independent, whole-night features, could discriminate subjects with and without PTSD at an individual level. First, we divided the study data into a *training* set, consisting of the first 47 consecutive subjects in the study (18 with PTSD), and a *testing* set, consisting of the next 31 subjects (13 with PTSD). Then, using only the *training* set, we identified three uncorrelated EEG features that discriminated subjects with and without PTSD in each of the two nights of the study (high-delta power in the C3 channel, phase synchrony between the C4 and P3 channels in the high-alpha band, and phase synchrony between the C4 and F3 channels in the low-gamma band). Using these features, we developed a logistic regression model based on the subjects from the *training* set. Then, to independently assess the performance of this model, we computed the value of the three features for each

TABLE 3 | Area under the receiver operating characteristic curve (AUC) for a logistic regression model consisting of the three features shown in **Table 4** and developed by combining data from both nights of the *training* set.

	Training	Testing	
		Night 1	Night 2
AUC	0.83 (0.73, 0.92)	0.84 (0.70, 0.98)	0.80 (0.64, 0.96)
	Threshold = 0.37 (Training Sen. = 0.81)		
Sen.	0.81	0.62	0.54
Spe.	0.74	0.89	0.67
Adj. PPV	0.35	0.50	0.22
	Threshold = 0.26 (Training Sen. = 0.92)		
Sen.	0.92	0.85	0.85
Spe.	0.57	0.67	0.67
Adj. PPV	0.27	0.31	0.31

Values within parentheses indicate 95% confidence intervals.
Adj. PPV = [sensitivity × prevalence] / [(sensitivity × prevalence) + {(1 - specificity) × (1 - prevalence)}].
The sensitivity (Sen.), specificity (Spe.), and adjusted positive predictive value (Adj. PPV) for a PTSD prevalence of 15% at two different thresholds of the model output (corresponding to training-set sensitivities of 0.81 and 0.92) are shown above.

TABLE 4 | Area under the receiver operating characteristic curve (AUC) for each of the three features used in the logistic regression model.

Feature	Training set [*n* = 47 (18 PTSD)]	Test set [*n* = 31 (13 PTSD)]	
		Night 1	Night 2
LP-C3-Hδ			
AUC	**0.71** (0.60, 0.82)	0.63 (0.42, 0.84)	0.60 (0.38, 0.82)
	Threshold = +1.28 (Training Sen. = 0.92)		
Sen.	0.92	0.77	0.77
Spe.	0.26	0.28	0.33
Adj. PPV	0.18	0.16	0.17
W-C4-P3-Hα			
AUC	**0.74** (0.63, 0.84)	0.55 (0.34, 0.77)	0.64 (0.42, 0.86)
	Threshold = −1.62 (Training Sen. = 0.92)		
Sen.	0.92	1.00	0.92
Spe.	0.26	0.50	0.33
Adj. PPV	0.18	0.26	0.20
W-C4-F3-Lγ			
AUC	**0.73** (0.61, 0.84)	0.67 (0.47, 0.88)	**0.74** (0.57, 0.92)
	Threshold = −2.23 (Training Sen. = 0.92)		
Sen.	0.92	0.85	1.00
Spe.	0.19	0.11	0.06
Adj. PPV	0.17	0.14	0.16

Values within parentheses indicate 95% confidence intervals (CIs). Bold values indicate statistical significance (lower bound of the CI > 0.50).
Adj. PPV = [sensitivity × prevalence] / [(sensitivity × prevalence) + {(1–specificity) × (1–prevalence)}].
For each feature, the sensitivity (Sen.), specificity (Spe.), and adjusted positive predictive value (Adj. PPV) for a PTSD prevalence of 15% at a training-set sensitivity of 0.92 are shown above the AUC values.

of the subjects in the *testing* set, and used the model to classify the 31 subjects in this set into PTSD or non-PTSD. Assessment of the logistic regression model on the *testing*-set data resulted in AUCs above 0.80 for each of the two consecutive nights, a high sensitivity (0.85), a moderate specificity (0.67), and an adjusted PPV of 0.31, which is twice the prevalence of PTSD in combat-exposed Veteran men (**Table 3**). This means that if the model classifies a combat-exposed Veteran man as having PTSD, the probability that the subject actually has this disorder is twice as large as a random-choice selection.

Interpretation of the Three Selected Features

The three features used in the logistic regression model were from the delta-, alpha-, and gamma-band clusters (**Figure 3**; clusters 1, 6, and 4, respectively). Of these, the log powers in the C3 channel in both low- and high-delta bands (**Figure 3**; clusters 1) were smaller in subjects with PTSD compared to those without PTSD (**Supplementary Table 1** shows the effect sizes for each feature, with negative effect sizes indicating lower feature values in PTSD.) These results are similar to those of our prior study, which showed that delta power during NREM is smaller in subjects with PTSD compared to those without PTSD (6). This is not surprising because, as noted in Methods section Feature Computation, sleep predominantly consists of the NREM stage 2 (**Table 2**) and, hence, any feature based on whole-night, stage-independent averages will contain the most information for this sleep stage. Given that delta power indicates sleep depth (29), it is likely that lower delta power in subjects with PTSD indicates disturbed sleep.

The first of the two synchrony features, W-C4-P3-Hα, was the representative of the alpha-band cluster (**Figure 3**, cluster 6), which mainly consisted of synchronies between EEG channels located on the left hemisphere, save for two synchrony pairs that involved the C4 channel. The synchronies in this cluster were larger in subjects with PTSD compared to those without PTSD (**Supplementary Table 1**). This is also in line with our prior findings, in which subjects with PTSD showed larger alpha synchrony than subjects without PTSD in the left fronto-parietal regions during NREM sleep (8). The second feature, W-C4-F3-Lγ, was the representative of the gamma-band cluster (**Figure 3**, cluster 4), which mainly consisted of cross-hemisphere synchronies between the frontal and central channels that were larger in subjects with PTSD (**Supplementary Table 1**). Although the increased synchrony in these bands may reflect impaired sleep processes in PTSD, focused research on this topic will be needed before we can make any conclusive statements regarding the specific underlying neurophysiological mechanisms of these features.

Model Evaluation Procedure

In general, there are two main approaches to evaluate the performance of classification models. One approach is cross-validation, which entails multiple rounds of model development and evaluation on different partitions of the study data. The other involves splitting the data into a *training* set and a *test* set at the outset. The former approach allows for the use of the entire dataset for model development, but because each subject is used in model development in at least one of the cross-validation

rounds, its ability to truly assess model performance on unseen subjects may be reduced. Although the latter approach decreases the sample size available for model development, it allows for independent evaluation of model performance. In this work, we used this approach because it more closely mimics how the results in one study are subsequently validated in future studies using a completely different set of subjects.

Limitations of the Study

A limitation of this work is that our study population excluded subjects with PTSD who had comorbid sleep disorders, such as depression (30) or insomnia (31), which share symptoms with PTSD. To test whether the features included in the logistic regression model are specific to PTSD, we would need to test the model in two different populations: one that included sleep disorders other than PTSD and another that included subjects with PTSD and comorbidities. If the features were specific to PTSD, then the model performance would be degraded in the first population and improved in the second. However, the model performance in the second population should not be as good as those of the present study population, which consisted of comorbidity-free subjects with PTSD.

Another potential limitation relates to the use of whole-night averages of the EEG features rather than an approach that considers the time-series nature of the EEG signal. By averaging the features across the entire 8 h of time in bed, it is possible that our analysis excluded alterations in the power or synchrony features in short-length sleep stages, e.g., during REM or NREM stage 3 sleep. However, analyzing time-series features in a naïve manner, i.e., by assuming that the feature value at one time point is independent of that at another time point, could increase the chance of false associations when sample sizes are small, as was the case in this study. A robust analysis of time-series features would require identification of temporal patterns in each feature (32) and, hence, a more elaborate methodology whose results would likely be difficult to compare with other studies.

CONCLUSION

In this work, we assessed the ability of a multivariate classifier to diagnose PTSD at an individual level, using whole-night, stage-independent features to obviate the need for laborious manual scoring of sleep. After identifying univariate features associated with PTSD, we combined them into a logistic regression model to test whether the model could discriminate subjects with and without PTSD. We developed the model on an initial *training* set from consecutive subjects enrolled in the study, and then evaluated its performance on a separate, independent *test* set from subsequent subjects. Performance on the *test* set yielded AUCs above 0.80 for each of the two consecutive nights, high sensitivity (0.85), and an adjusted PPV that is twice the prevalence of PTSD in combat-exposed Veteran men. These findings imply that, if the model predicts that a subject has PTSD, the likelihood of that subject actually having PTSD is twice the underlying prevalence rate. Thus, the model provides an objective means to more accurately identify individuals with this disorder.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by University of Pittsburgh Institutional Review Board (Pittsburgh, PA) The U.S. Army Medical Research and Development Command Human Research Protection Office (Ft. Detrick, MD). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

SL, CW, and JR conceived the research idea and study objectives. SL performed all analyses reported in the study. SL and TO wrote the manuscript. CW and JR provided inputs for improving the analysis and edited the manuscript. JC and AG performed the laboratory study. All authors read and approved the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2020.532623/full#supplementary-material

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Aberrant Hippocampal Network Connectivity Is Associated With Neurocognitive Dysfunction in Patients With Moderate and Severe Obstructive Sleep Apnea

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Objectives: This work aims to explore the changes of functional connectivity (FC) within the hippocampus network in patients with moderate and severe obstructive sleep apnea (OSA) and its correlation with neurocognitive dysfunction to explore the potential neurophysiological mechanism.

Methods: A total of 32 treatment-naïve patients with moderate or severe OSA and 26 healthy controls (HCs), matched in age, gender, and education, underwent the evaluations of Epworth Sleep Scale, neurocognitive function, full-night polysomnography, and resting-state functional magnetic resonance imaging. The FC map of the hippocampus to other brain areas was compared among 15 OSA patients and 15 HCs with little head motion. Finally, the correlation between hippocampus FC strength and respiratory sleep parameters and neurocognitive assessments was analyzed.

Results: Compared with HCs, the right hippocampus showed a significantly decreased FC with the bilateral insular lobe, right thalamus, and right anterior cingulate gyrus (ACG) and an increased FC with the right superior and middle temporal gyrus, left posterior cingulate gyrus, and left angular gyrus in the patients with OSA. The left hippocampus presented a significantly decreased FC with the left anterior cerebellum in patients with OSA. In addition, the aberrant right hippocampal FC with the right ACG was significantly correlated with disease severity and disrupted sleep architecture in the OSA group. Furthermore, after adjusting the related confounding factors, the FC strength between the right hippocampus, right insular lobe, and right thalamus was positively associated with the scores of Stroop Color–Word Test (SCWT) or Hopkins Verbal Learning Test—Revised (HVLT-R), while the FC between the right hippocampus and the right middle temporal gyrus was negatively correlated with the scores of HVLT-R. The right hippocampus FC with right superior temporal gyrus, left angular gyrus, and ACG were all negatively related to the scores of the symbol coding test ($r = -0.642$, $p = 0.045$; $r = -0.638$, $p = 0.047$;

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$r = -0.753$, $p = 0.012$), respectively. The FC between the left hippocampal and the left anterior cerebellar lobe showed a positive relationship with the scores of HVLT-R ($r = 0.757$, $p = 0.011$) and CPT-3D ($r = -0.801$, $p = 0.005$).

Conclusion: The hippocampus presented abnormal FC with the cerebral and cerebellar regions extensively in OSA, and the correlation between abnormal hippocampal network FC and neurocognitive dysfunction in OSA suggests a promising insight to explore the potential biomarker and pathophysiologic mechanism of neurocognitive dysfunction of OSA.

Keywords: obstructive sleep apnea, neurocognitive impairment, hippocampus, functional connectivity, cerebellum, default mode network

BACKGROUND

Obstructive sleep apnea (OSA) syndrome is one of the commonest types of sleep disorder, of which the prevalence of moderate-to-severe OSA in the population is high, with up to 49.7% in men and 23.4% in women (1). Repetitive partial or complete obstruction of the upper airway during sleep leads to two prominent pathophysiological characteristics of OSA: nocturnal intermittent hypoxia and sleep fragmentation/disruption (1). More importantly, chronic intermittent hypoxia and sleep disruption would cause a large amount of production in reactive oxygen species, inducing excessive activation of oxidative stress responses, including lipid peroxidation, protein oxidation, and DNA oxidation, and leading to dysfunction of the mitochondria, endoplasmic reticulum, and endothelial cell, plus massive inflammatory responses (2, 3). The brain, especially the cerebral cortex and hippocampus, is extremely vulnerable to hypoxia and oxidative stress, and the above-mentioned pathophysiological changes in the brain of OSA patients could induce the overproduction of neuroinflammatory cytokines and cellular dysfunction, resulting in chronic damage and even apoptosis of neuronal cells, and eventually lead to neurocognitive dysfunction (3). Neurocognitive impairment is a frequent clinical complaint from patients with OSA, which involves declined memory, attention/vigilance deficit, impaired executive function, etc. (4, 5).

In the recent decades, neuroimaging technologies, including functional magnetic resonance imaging (fMRI), voxel-based morphometry, magnetic resonance spectroscopy, diffusion tensor imaging, *etc*., were introduced in studies of OSA to investigate the changes of brain function and structures and explore the potential neuropathologic mechanism of neurocognitive impairment in OSA (6–9). The previous structure MRI studies found decreased white matter integrity and volume (10, 11), regional cortical thinning (12), altered gray matter volume and density (6, 13), reduced mean diffusivity (9), and abnormal cerebral metabolisms, such as regional reduced N-acetyl aspartate/choline ratios and choline/creatine ratios, lower γ-aminobutyric acid, and higher glutamate (7, 14), among OSA patients. Resting-state fMRI (rs-fMRI), an advanced fMRI technology to evaluate brain activity in the spontaneous state, has demonstrated abnormality in regional homogeneity (15), global and regional functional connectivity (FC) (16), and amplitude of low-frequency fluctuation (17) within individuals with OSA. rs-fMRI is a useful tool to detect the changes of brain functional activities in neurodegenerative diseases (18, 19), in which seed-based FC is a widely applied approach to evaluate the functional synchronicity between a specific region of interest and the rest of the brain voxels by calculating the correlation coefficients of blood oxygen level-dependent time series signals among these brain regions (20). Abnormal FC was found in multiple brain regions including the insula (16, 21), amygdala sub-regions (22), prefrontal cortex (23), and default-mode network (24–26) in OSA patients.

In recent years, more and more evidence demonstrated that OSA was an important risk factor for Alzheimer's disease (AD) (27, 28). AD is an irreversible neurodegenerative disorder characterized by a progressive decline in memory and other neurocognitive functions, including visual–spatial skills, attention, executive function, decision-making ability, language ability, personality and behavioral abnormality, *etc*. (29) Classical cerebrospinal fluid biomarkers of AD, such as increased tau proteins, reduced β-amyloid$_{42}$, and elevated lactate levels, were demonstrated in the cerebrospinal fluid of individuals with OSA (30, 31). OSA also is involved in up-regulating the phosphorylation of tau proteins, promoting the production of β-amyloid$_{42}$, and enhancing synaptic dysfunction, which indicate that similar pathophysiological alterations in the brain existed between AD and OSA (27). It is widely acknowledged that the hippocampus is one of the brain regions with the most prominent pathological lesions in patients with AD (29). The hippocampus is a pivotal and fundamental brain area responsible for neurogenesis and function of dentate gyrus and hippocampal circuitry, playing an important role in the process of learning and memory, including sensory memory, short-term memory, and long-term memory (32). Besides these, the hippocampus is vulnerable to hypoxia, oxidative stress, and inflammation, and these pathological responses are recognized as classical pathological manifestations of OSA (2). A brain MRI study conducted by Torelli and colleagues demonstrated that the total volume of the hippocampus was reduced in patients with moderate–severe OSA and was correlated with scores of the tests of verbal memory and executive function (33). However, little is

known about hippocampal FC and its relationship with different fields of neurocognitive function in patients with OSA.

In this study, MATRICS Consensus Cognitive Battery and Stroop Color–Word Test (SCWT) were employed to evaluate the neurocognitive impairment in patients with moderate to severe OSA, including multiple cognitive fields in information processing speed, memory, attention/alertness, and executive function. Furthermore, the FC of the hippocampus network was measured by the method of seed point correlation analysis, and the correlation between FC strength and respiratory–sleep parameters and neurocognitive function was further analyzed.

MATERIALS AND METHODS

Subjects

A total of 32 treatment-naïve, newly diagnosed patients with moderate to severe OSA [apnea hypopnea index (AHI) >15 events/h) from the respiratory and sleep center of The Second Xiangya Hospital, Central South University, and 26 age-, sex- and education years-matched healthy controls (HCs) were recruited in this study (**Table 1**). The diagnosis criteria of OSA referred to the guideline published by American Academy of Sleep Medicine in 2014 (34). The exclusion criteria for all subjects were as follows: [1] clinical history of heart disease, neurological disorder, psychiatric illness, other respiratory or sleep disorders, malignant tumors, drug and alcohol abuse, recent surgery, trauma, infection, or other system diseases, *etc*., [2] received the same or similar cognitive tests before, and [3] contraindicated for MRI. The inclusion criteria for HCs were as follows: [1] good sleepers, no snoring or apnea during sleep, [2] AHI <5 (confirmed by followed polysomnography), [3] body mass index (BMI) >24 kg/m^2, and [4] without neurological diseases that can potentially influence the results of neurocognitive function and fMRI. This research protocol was approved by the Ethics Committee of The Second Xiangya Hospital, Central South University, and all subjects were informed of the study details and have provided their consent before participation.

Assessment of Sleepiness, Neurocognitive Function, and Polysomnography

Daytime sleepiness was evaluated by the Epworth Sleep Scale (ESS), and neurocognitive function was assessed by the MATRICS Consensus Cognitive Battery and SCWT when the subjects got admitted. Seven domains of neurocognitive function were assessed in this study, including speed of information processing, attention/vigilance, working memory, executive function, short-term memory in verbal learning, ability to reasoning, and problem solving. The full-night polysomnography (Embla S4000; Medcare Technologies, Fuquay Varina, NC, USA) was performed on the first night. The detailed process of neurocognitive evaluation has been shown in our previously published paper (35). All the above-mentioned assessments were uniformly conducted by a professionally trained physician who was blinded to the clinical information of subjects. All the examinations were performed in the same order.

TABLE 1 | Comparison of characteristics in demography, clinical and sleep parameters, and neurocognitive tests between obstructive sleep apnea (OSA) group and HC group.

Characteristics	OSA (n = 32)	HC (n = 26)	p
Age (year)[a]	43.44 ± 11.14	42.77 ± 12.67	0.832
Men/women[c]	28/4	20/6	0.062
Education (year)[b]	12 (9–14)	11 (9–16)	0.762
BMI (kg/m^2)[a]	28.87 ± 3.29	27.18 ± 1.68	0.015*
Smoking index[b]	65 (0–400)	0 (0–425)	0.402
Drinking index[b]	0 (0–825)	0 (0–250)	0.565
ESS scores[b]	13.78 ± 4.72	8.31 ± 4.23	<0.001**
Nocturnal SP (mmHg)[a]	135.94 ± 14.62	127.54 ± 14.57	0.034*
Nocturnal DP (mmHg)[b]	88.5 (81.8–93.8)	78 (72.0–83.5)	<0.001**
Heart rate (beats/min)[a]	94.3 ± 11.68	76.46 ± 7.61	<0.001**
AHI (events/h)[b]	60.40 ± 21.2	2.34 ± 1.45	<0.001**
ODI (events/h)[b]	61.75 (38.5–79.5)	1.65 (0.67–2.92)	<0.001**
$LSaO_2$ (%)[b]	62.5 (54.5–75.0)	91 (88.3–93.0)	<0.001**
$MSaO_2$ (%)[b]	94 (87.3–95.0)	96 (95–97)	<0.001**
Total sleep time (min)[b]	370.8 (286.0–455.8)	411.8 (311.5–435.5)	0.684
Sleep efficiency (%)[a]	66.1 ± 21.34	69.44 ± 12.17	0.457
N1 stage/TST (%)[b]	20.9 (12.9–30.0)	18.5 (10.5–27.1)	0.321
N2 stage/TST (%)[a]	54.87 ± 17.20	51.36 ± 11.87	0.396
Light sleep/TST (%)[b]	85.6 (68.3–93.3)	71.4 (62.4–81.6)	0.011*
N3 stage/TST (%)[b]	7.5 (0.5–24.7)	19.7 (8.4–25.8)	0.139
REM/TST (%)[b]	4.1 (1.7–7.5)	11.65 (6.68–15.15)	<0.001**
Mean head motion (cm)[b]	0.151 (0.08–0.24)	0.086 (0.08–0.113)	0.07**
TMT-A[a]	44.69 ± 15.39	33.62 ± 8.85	0.037*
HVLT-R[b]	20 (15–23)	24 (19–28)	0.015*
WMS-III: spatial span[b]	14.5 (12–17)	17 (14–19)	0.016*
NAB: mazes[a]	13.34 ± 5.88	17.08 ± 4.80	0.012*
Category fluency test[a]	19.7 ± 6.2	24.7 ± 6.6	0.004**
SCWT—word[b]	85 (63–93)	88 (80–100)	0.049*
SCWT—color–word[b]	28 (20–40)	41 (31–48)	0.003**

HC, healthy control; AHI, apnea–hypopnea index; ODI, oxygen desaturation index; $LSaO_2$, lowest oxygen saturation; $MSaO_2$, mean oxygen saturation; BMI, body mass index; ESS, Epworth Sleepiness Scale; SP, systolic pressure; DP, diastolic pressure; TST, total sleep time; REM, rapid eye movement; TMT-A, Trail Making Test: part A; HVLT-R, Hopkins Verbal Learning Test—Revised; WMS-III, Wechsler Memory Scale-III; NAB, Neurological Assessment Battery: mazes; SCWT, Stroop Color–Word Test.

Sleep efficiency is calculated by dividing total sleep time by total bedtime.

**p < 0.05; **p < 0.01.*

[a] *Student's t-test (data are shown as mean ± standard deviation).*

[b] *Mann–Whitney U-test [data are shown as median (interquartile range)].*

[c] *Chi-square test (data are presented as number of people).*

rs-fMRI Data Acquisition

All the subjects underwent resting-state functional MRI on a Philips 3.0 Tesla MRI scanner. The participants were asked to wear headphones to decrease scanner noise and minimize head motion, breathe calmly, keep their eyes closed, and stay motionless without any specific thoughts. The functional image data were obtained by an echo-planar imaging (EPI) sequence, and the parameters were as follows: TR/TE = 2,000/30 ms, 33 slices, 64 × 64 matrix, 22 × 22 cm^2 field of view, 90° flip angle, 0.4 cm thickness, and 0.6 mm gap. Each functional MRI scanning took 8 min and 8 s, and 240 volumes (total of 7,920 images) were acquired.

Data Preprocessing

The Matlab-based Statistical Parametric Mapping (SPM8) software (Wellcome Department of Imaging Neuroscience, London, UK) was used to preprocess the rs-fMRI imaging data. To avoid the impact of interference signal brought by the initial adaptation of the magnetic environment, the first five brain volumes of the rs-fMRI were discarded, and the remaining 235 volumes were preprocessed as follows: [1] Firstly, correction of slice-timing and head motion was conducted. The subjects with head translation in any cardinal direction (x, y, z) of more than 1.5 mm were excluded. Finally, 15 OSA patients and 15 HCs were selected for further analysis of the difference of FC among the hippocampus network (**Table 2**); [2] Secondly, spatial normalization of images was performed in a standard EPI template of the Montreal Neurological Institute, and then the images were resampled to $3 \times 3 \times 3$-mm^3 voxels; [3] Thirdly, the standardized functional images were spatially smoothened with a 6-mm full-width at half-maximum Gaussian kernel and temporally band pass-filtered (0.01–0.08 Hz); and [4] Lastly, a linear regression was performed to decrease the influence of spurious covariates and signals from low-frequency synchronous oscillation.

Analysis of FC Among Hippocampal Networks

The bilateral hippocampus was defined as the seed region of interest in accordance with the automated anatomical labeling template generated by Rest 1.8 software (http://www.restfmri.net/forum/REST_V1.8) (36). The left and the right hippocampus were selected as two seeds, and FC coefficients were calculated within the whole brain. The average blood oxygen level-dependent time series of the seed region of interest was extracted from each subject, and then the Pearson correlation coefficient was computed between the time series of the seed and the time series of each voxel in the whole brain. The FC value of each voxel in the whole brain represented the FC of this voxel with the seed region of interest, and the correlation coefficients were transformed into Z values by using Fisher Z-score to bring the results closer to a normal distribution. Two independent-sample Student's t-tests were performed to identify the brain regions that show a significantly different functional connection with the hippocampus between the OSA group and the HCs group, and the brain region with $p < 0.001$ and cluster volume > 10 voxels was defined as a meaningful brain region with significant FC difference in the hippocampus network after AlphaSim correction. Finally, the significantly different brain regions' images were presented by using the XjView software (http://people.hnl.bcm.tmc.edu/cuixu/xjView). In addition, the REST software was used to extract the FC strength between the peak point of the abnormal FC brain areas of each patient and the hippocampus, the correlation analysis between the aberrant FC coefficients and sleep–breathing parameters, and the scores of neurocognitive function tests within the OSA group.

Statistical Analysis

Statistical analysis was conducted by using the SPSS 23.0 statistical software (IBM Corp., Armonk, NY, USA). Kolmogorov–Smirnov test was performed to evaluate the normality of data. Student's t-test was employed in the normally distributed data, and Mann–Whitney U-test was used to analyze the non-normally distributed data to compare the data difference among the OSA group and HCs group. Partial correlation analysis was used in all the correlation analyses by adjusting the potential confounding effects, including age, BMI, and education years, to analyze the relationship between abnormal hippocampal FC, sleep–breathing parameters, and neurocognitive impairment in the OSA group. $P < 0.05$ was considered significantly different. Double-sided test was employed among all data analysis.

RESULTS

Demographic, Clinical, and Neurocognitive Function Data

As shown in **Table 1**, no significant differences were shown in gender, age, education years, smoking index, and drinking index between the OSA group ($n = 32$) and HCs group ($n = 26$). As expected, significant differences were found in body mass index, AHI, oxygen desaturation index (ODI), mean oxygen saturation ($MSaO_2$), lowest oxygen saturation ($LSaO_2$), and scores of ESS among these two groups. Significantly elevated nocturnal systolic blood pressure, diastolic blood pressure, and heart rate were also observed in patients with OSA. In addition, patients with OSA

TABLE 2 | Correlation between sleep structure and neurocognitive tests among obstructive sleep apnea group [adjusting for age, education, body mass index (BMI), smoking years, and alcohol years].

$n = 32$		TST (min)	Sleep efficiency (%)	LS/TST (%)	REM/TST (%)
TMT-A	$r =$	0.010	−0.017	−0.228	0.413
	$p =$	0.959	0.931	0.253	0.032*
HVLT-R	$r =$	−0.118	−0.122	−0.410	0.228
	$p =$	0.557	0.544	0.033*	0.252
Category fluency	$r =$	−0.489	−0.501	0.164	0.138
	$p =$	0.010*	0.008**	0.415	0.492

The data were obtained by partial correlation analysis by adjusting the confounding factors of age, education, BMI, smoking index and drinking index; TST, total sleep time; REM, rapid eye movement; LS, light sleep; TMT-A, Trail Making Test: part A; HVLT-R, Hopkins Verbal Learning Test—Revised.
**p < 0.05; **p <0.01.*

presented increased light sleep (LS) and decreased N3 sleep and rapid eye movement (REM) sleep. No significant differences were found in total sleep time, sleep efficiency, and N1 sleep and N2 sleep between the OSA group and the HCs group. Besides these, compared with HCs, significantly increased head motion was presented in the OSA group. Furthermore, the OSA patients showed poorer performance in the multiple neurocognitive tests including Trail Making Test A (TMT-A), Hopkins Verbal Learning Test—Revised (HVLT-R), Wechsler Memory Scale-III: spatial span (WMS-IIISS), mazes, category fluency, Continuous Performance Test (CPT), and SCWT ($p < 0.05$).

Correlation Between Sleep Structure and Blood Pressure, Neurocognitive Tests in Patients With OSA

Among the OSA group, after adjusting the confounding factors of age, education years, BMI, smoking index, and drinking index, the proportion of REM in TST (REM%TST) was positively associated with the scores of the TMT-A test ($r = 0.413$, $p = 0.032$), and the percentage of light sleep in TST (LS%TST) was negatively correlated with the scores of HVLT-R ($r = -0.410$, $p = 0.033$). The scores of the category fluency test were negatively correlated with TST ($r = -0.489$, $p = 0.010$) and sleep efficiency ($r = -0.501$, $p = 0.008$) (shown in **Table 2**, **Figure 1**).

Alterations of FC Among Hippocampal Network in Patients With OSA

The demographic characteristics of the subjects included in the analysis of hippocampal network FC are shown in **Table 3**. There was no significant difference in age, gender distribution, education years, BMI, smoking index, and drinking index between the OSA group and the HCs group. Compared with HCs, the right hippocampus presented a significantly reduced FC with the bilateral insular lobes, right thalamus, and anterior cingulate gyrus (ACG), while a significantly increased FC was observed with right middle temporal gyrus (RMTG), right superior temporal gyrus (RSTG), left posterior cingulate gyrus

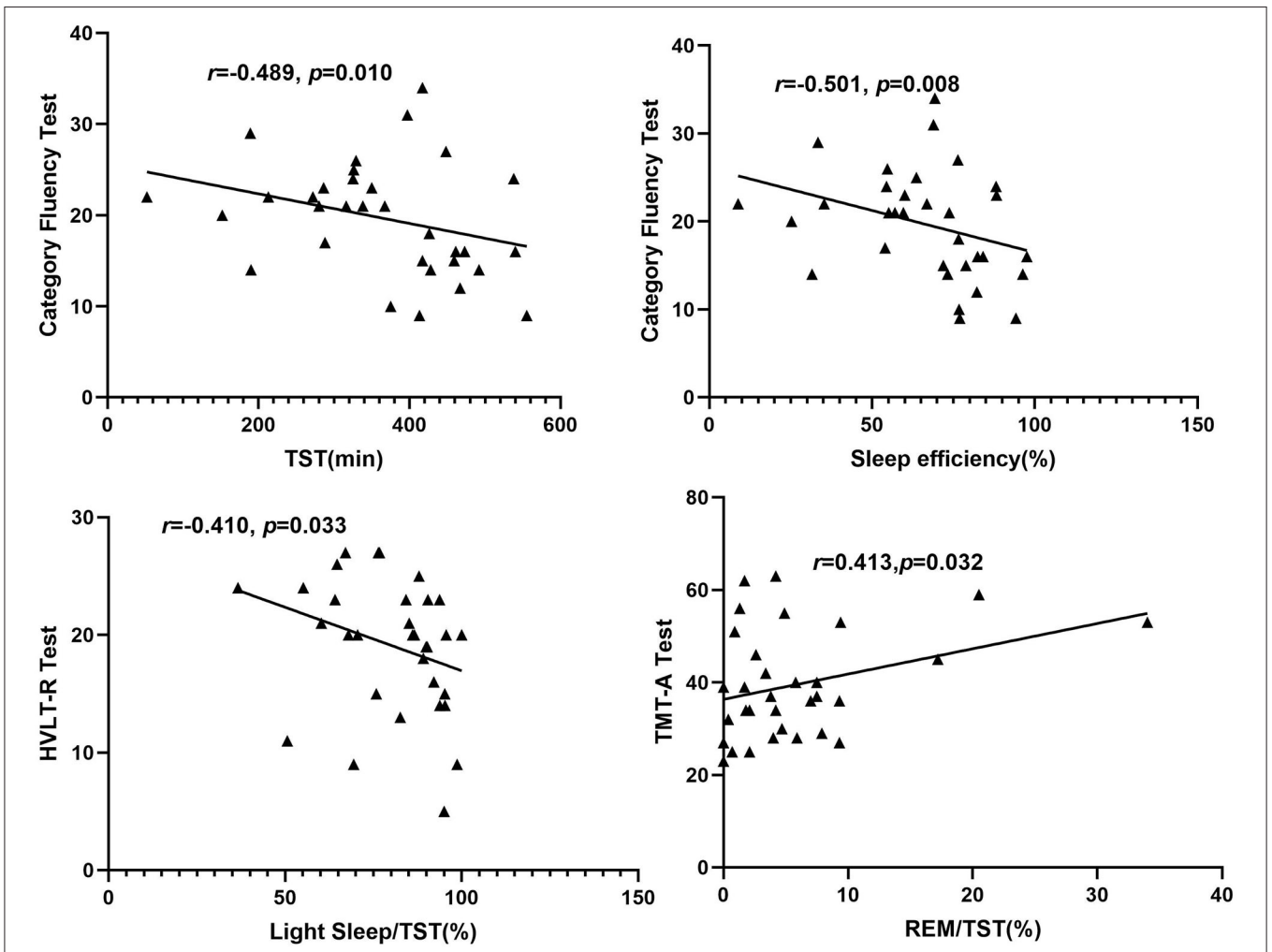

FIGURE 1 | Correlation between sleep structure and neurocognitive tests among patients in the obstructive sleep apnea group. REM, rapid eye movement; TMT-A, Trail Making Test: part A; HVLT-R, Hopkins Verbal Learning Test—Revised.

TABLE 3 | Basic clinical characteristics of the subjects included in the functional connectivity analysis.

	Obstructive sleep apnea ($n = 15$)	HC ($n = 15$)	*P*
Age (year)[a]	42.87 ± 12.17	39.67 ± 12.52	0.484
Men/women[c]	13/2	12/3	0.775
Education (year)[b]	12 (9–16)	15 (9–16)	0.461
BMI (kg/m^2)[a]	28.44 ± 3.38	27.16 ± 1.57	0.199
Smoking index[b]	0 (0–400)	0 (0–300)	0.838
Drinking index[b]	0 (0–500)	0 (0–0)	0.902
ESS scores[a]	12.4 ± 3.62	8.14 ± 4.03	0.006**
Nocturnal SP (mmHg)[a]	138.9 ± 16.31	124.8 ± 8.87	0.006**
Nocturnal DP (mmHg)[b]	92 (92–102)	75 (70–78)	<0.001**
Heart rate (beats/min)[a]	92.9 ± 12.55	77.1 ± 8.41	<0.001**
AHI (events/h)[a]	58.9 ± 22.74	2.17 ± 1.26	<0.001**
ODI (events/h)[a]	52.9 ± 25.21	1.61 ± 0.93	<0.001**
$LSaO_2$ (%)[a]	67.2 ± 14.41	90.6 ± 3.06	<0.001**
$MSaO_2$ (%)[b]	92 (94–96)	96 (95–97)	0.005**
Total sleep time (min)[b]	328.5 (190–447.5)	420 (333.5–432.5)	0.217
Sleep efficiency (%)[a]	58.1 ± 24.5	73.98 ± 8.45	0.029*
N1 stage/TST (%)[a]	32.6 ± 20.28	21.4 ± 11.81	0.075
N2 stage/TST (%)[a]	53.3 ± 19.24	50.3 ± 16.71	0.647
Light sleep/TST (%)[b]	58.1 ± 24.5	69.3 ± 10.56	0.120
N3 stage/TST (%)[b]	4.4 (0.3–11.8)	21.2 (10.8–24.5)	0.106
REM/TST (%)[b]	3.8 (1.3–7.5)	11.8 (5.4–18.7)	0.007**
Mean head motion (cm)[a]	0.1 ± 0.0315	0.078 ± 0.015	0.021*

*HC, healthy control; AHI, apnea–hypopnea index; ODI, oxygen desaturation index; $LSaO_2$, lowest oxygen saturation; $MSaO_2$, mean oxygen saturation; BMI, body mass index; ESS, Epworth Sleepiness Scale; SP, systolic pressure; DP, diastolic pressure; TST, total sleep time; REM, rapid eye movement. *$p < 0.05$ and **$p < 0.01$.*

[a]*Student's t-test (data are shown as mean ± standard deviation).*

[b]*Mann–Whitney U-test [data are shown as median (interquartile range)].*

[c]*Chi-square test (data are presented as number of people).*

(LPCG), and left angular gyrus (LAG) in patients with OSA (shown in **Table 4**, **Figure 2**). In addition, the left hippocampus showed a decreased FC with the left anterior cerebellum lobes (LACL) in patients with OSA (**Table 4**, **Figure 2**).

Correlation Between Changes of Hippocampal Network FC and Sleep Respiratory Parameters in Patients With OSA

As shown in **Table 5** and **Figure 3**, among the OSA group, after adjusting for the influence of age, education, BMI, smoking index, and drinking index, the FC strength between the right hippocampus and the right ACG was positively correlated with AHI ($r = 0.708$, $p = 0.022$), ODI ($r = 0.737$, $p = 0.015$), and N3 sleep %TST ($r = 0.778$, $p = 0.008$), respectively, but negatively correlated with mean SaO_2 ($r = -0.791$, $p = 0.006$) and light sleep %TST ($r = -0.893$, $p < 0.001$). The FC strength between the right hippocampus and the right thalamus was negatively associated with light sleep %TST ($r = -0.800$, $p = 0.005$). The FC strength between the right hippocampus and the LAG showed a positive correlation with ODI ($r = 0.652$, $p = 0.042$) and a negative correlation with N1 sleep %TST ($r = -0.719$, $p = 0.019$) (**Figure 4**). The FC strength between the right hippocampus and RMTG was negatively associated with REM sleep %TST ($r = -0.720$, $p = 0.019$) and the scores of ESS ($r = -0.650$, $p = 0.042$). The FC strength between the right hippocampus and LPCG was positively associated with light sleep %TST ($r = 0.761$, $p = 0.011$) while negatively associated with N3 sleep %TST ($r = -0.641$, $p = 0.046$). The FC strength between the right hippocampus and RSTG was positively related to REM sleep %TST ($r = 0.712$, $p = 0.021$). In addition, the FC between the left hippocampus and LACL was positively related to REM sleep %TST ($r = 0.817$, $p = 0.004$).

Correlation Between Changes of Hippocampal Network FC and Neurocognitive Dysfunction in Patients With OSA

As shown in **Table 6** and **Figure 5**, by adjusting the confounding variables of age, education, BMI, smoking index, and drinking index, the FC strength between the right hippocampus and the right insular lobe was positively associated with the scores of SCWT ($r = 0.688$, $p = 0.028$) within the OSA group. The FC strength between the right hippocampus and RMTG was negatively correlated with the scores of HVLT-R ($r = -0.661$, $p = 0.037$), while the FC strength between the right hippocampus and right thalamus was positively correlated with the scores of HVLT-R ($r = 0.858$, $p = 0.002$). The scores of the symbol coding test showed an inverse relationship with the FC strength between the right hippocampus and RMTG ($r = -0.642$, $p = 0.045$) and LAG ($r = -0.638$, $p = 0.047$), respectively. Moreover, for the left hippocampal FC, after adjusting the confounding factors of age, BMI, educational years, smoking index, and drinking index, the FC strength between the left hippocampus and LACL was positively related to the scores of HVLT-R ($r = 0.757$, $p = 0.011$) and CPT-3D test ($r = 0.801$, $p = 0.005$) (**Figure 6**).

DISCUSSION

FC Abnormality in the Hippocampal Network in OSA

The hippocampus, an important component of the limbic system, is located between the thalamus and the medial temporal lobe and plays a key part in learning, episode memory, and transformation of long-term memory (37); it is vulnerable to hypoxia and oxidative stress, which are important pathophysiological characteristics of OSA and thus may cause the impairment of synaptic plasticity and reduction of neurogenesis in the hippocampus (38). OSA is a significant risk factor and closely correlated with the onset of AD. The hippocampus is the commonest brain region suffering from the accumulation of β-amyloid$_{42}$ and neurofibrillary tangles (27). The previous studies have demonstrated that OSA patients showed reduced gray matter volume in the hippocampus which significantly increased after treatment (8, 39). In the current study, we found that, compared with HCs, the right hippocampus had an

TABLE 4 | Difference of FC between the hippocampus and other brain areas.

	Brain area	Brodmann area	Voxel	Montreal Neurological Institute atlas coordinates			*t*-value
				X	Y	Z	
Reduced FC	LIL	47	22	−36	15	−9	−4.85
	RIL	47	25	42	15	0	−4.10
	RT	–	44	18	−12	18	−5.05
	ACG	32	87	0	21	39	−4.86
	LACL	–	16	−3	−48	−24	−4.28
Increased FC	RMTG	21	18	60	−39	0	3.91
	LPCG	31	45	−6	−69	12	4.11
	RSTG	39	54	51	−63	15	4.77
	LAG	39	18	−48	−63	33	4.44

FC, functional connectivity; LIL, left insular lobe; RIL, right insula lobe; RT, right thalamus; ACG, anterior cingulate gyrus; LACL, left anterior cerebellar lobe; RMTG, right middle temporal gyrus; LPCG, left posterior cingulate gurus; RSTG, right superior temporal gyrus; LAG: left angular gyrus.

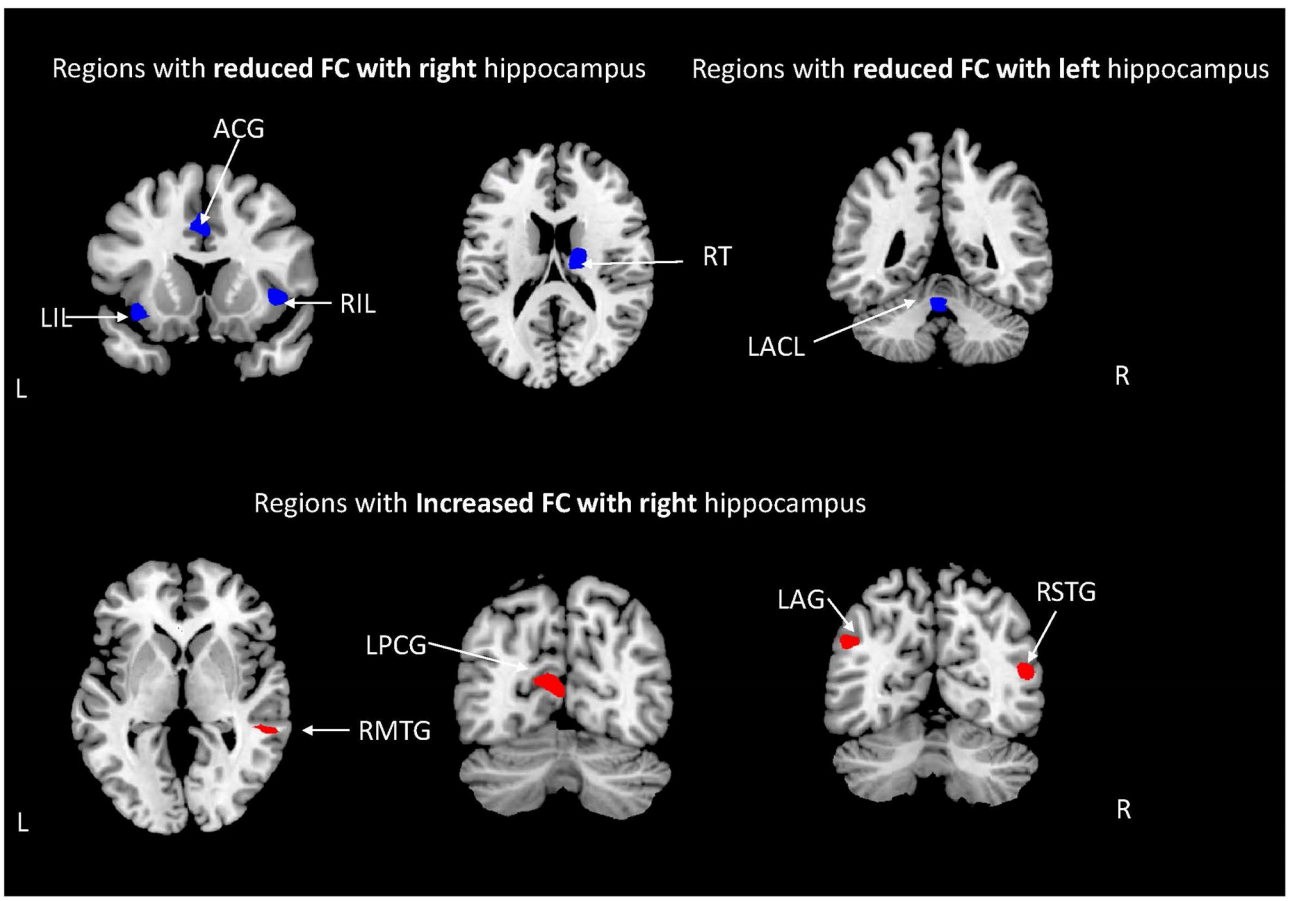

FIGURE 2 | The brain regions with abnormal hippocampal functional connectivity (FC) in patients with moderate to severe obstructive sleep apnea [weakened FC (blue), enhanced FC (red); AlphaSim corrected; $P < 0.001$; cluster volume > 10 voxels].

extensively abnormal FC with the cerebral cortex, sub-cortex, and cerebellum, including the bilateral insular, right thalamus, cingulate gyrus, right temporal gyrus, and angular gyrus, in patients with moderate-to-severe OSA, and a significantly reduced FC was shown between the left hippocampus and the left anterior cerebellar lobe in the subjects with OSA. In a previous study, Song and his colleagues also found that the right hippocampus showed reduced FC with the bilateral

TABLE 5 | Correlation between hippocampus functional connectivity and sleep respiratory parameters among patients in the obstructive sleep apnea group.

$n = 15$		RMTG	LPCG	RT	RSTG	LAG	ACG	LACL
AHI	$r =$	−0.080	−0.101	0.295	0.277	0.536	0.708	0.308
	$p =$	0.826	0.781	0.409	0.438	0.110	0.022*	0.387
ODI	$r =$	−0.023	−0.083	0.403	0.272	0.652	0.737	0.219
	$p =$	0.950	0.820	0.248	0.438	0.042*	0.015*	0.543
$MSaO_2$	$r =$	0.239	0.416	−0.571	0.044	−0.467	−0.791	−0.265
	$p =$	0.506	0.232	0.085	0.904	0.174	0.006*	0.459
ESS	$r =$	−0.650	−0.043	0.323	−0.155	0.167	0.108	−0.155
	$p =$	0.042*	0.905	0.363	0.670	0.644	0.767	0.670
N1/TST (%)	$r =$	−0.033	0.089	−0.595	−0.181	−0.719	−0.615	−0.103
	$p =$	0.928	0.806	0.070	0.617	0.019*	0.058	0.776
LS/TST (%)	$r =$	0.254	0.761	−0.800	−0.122	−0.265	−0.893	−0.518
	$p =$	0.479	0.011*	0.005**	0.736	0.459	<0.001**	0.125
N3/TST (%)	$r =$	0.118	−0.641	0.585	−0.260	0.282	0.778	0.115
	$p =$	0.745	0.046*	0.076	0.468	0.430	0.008**	0.751
REM/TST (%)	$r =$	−0.720	−0.344	0.522	0.712	0.009	0.353	0.817
	$p =$	0.019	0.330*	0.122	0.021	0.980	0.317	0.004

*The data were analyzed by the method of partial correlation analysis with adjustment of the confounding factors of age, education, body mass index, smoking index, and drinking index. AHI, apnea–hypopnea index; ODI, oxygen desaturation index; ESS, Epworth Sleep Scale; $MSaO_2$, mean oxygen saturation; TST, total sleep time; REM, rapid eye movement; N1, stage 1 sleep; N3, stage 3 sleep; LS, light sleep; SP, systolic pressure; LPCG, left posterior cingulate gyrus; RMTG, right middle temporal gyrus; RT, right thalamus; RSTG, right superior temporal gyrus; ACG, anterior cingulated gyrus; LAG, left angular gyrus; LACL, left anterior cerebellum lobe. *$p < 0.05$ and **$p < 0.01$.*

thalamus and para-hippocampal gyrus in patients with OSA and enhanced FC with precuneus and posterior cingulate gyrus (40) which, to some degree, were consistent with the findings in our study. The hippocampus, medial temporal lobe, cingulate gyrus, and angular gyrus are crucial components of default mode networks (DMN), which play an important role in regulating emotion, consciousness, memory, and introspection (24). The DMN consists of two spatially independent sub-networks: the anterior DMN and the posterior DMN. The anterior DMN is comprised of the medial prefrontal cortex, superior frontal gyrus, and anterior cingulate gyrus, and the posterior DMN includes the precuneus and posterior cingulate gyrus. Research indicated that the anterior DMN was responsible for emotional management and self-reference, while the posterior DMN mainly contributed to cognitive processing and memory retrieval (41). In this study, the right hippocampus showed weakened FC with the anterior DMN and increased FC with the posterior DMN in patients with OSA, which suggests a possible role of the connectivity difference between the hippocampus and the anterior and posterior DMN in the formation of neurocognitive deficits of OSA and is likely to be a potential neurocognitive mechanism in the development of cognitive dysfunction in OSA.

However, it should be noted that there was a significant difference both in the nocturnal systolic pressure and the diastolic pressure between OSA group and HCs group in the current study, which are common manifestations in OSA due to the interaction between nocturnal intermittent hypoxemia, hypercapnia, sleep fragmentation, neurohormonal dysregulation, and sympathetic activation (42). Recent studies demonstrated the impact of hypertension on cognitive impairment and FC alterations (43, 44). The study by Li and his colleagues indicated that impaired attention and executive function existed in patients with hypertension, and furthermore, altered FC in the frontoparietal networks mediated the effects of changes of white matter on the decreased executive function in patients with hypertension (43). Similarly, Carnevale et al. also found that the altered FC network was associated with cognitive impairment and brain microstructural injury in patients with hypertension (44). The pathogenesis of OSA and hypertension is interactional and bidirectional, in which OSA is a crucial risk factor of hypertension and hypertension is a very common complication in OSA (45). The prevalence of OSA in hypertension ranges from 30 to 83% (46), and the prevalence of hypertension in OSA is also high (30–70%) (47). Furthermore, cardiovascular morbidity in OSA could further aggravate neurocognitive impairment (48). Hypertension and OSA could cause abnormal metabolism and perfusion of the hippocampus and cortex and further trigger brain injuries and related pathological changes of Alzheimer's disease (27). Therefore, it is difficult to isolate the effects of hypertension on the changes of FC and cognitive impairment in OSA patients. The consequences of abnormal brain FC and abnormal neurocognitive functions are probably caused by the combined effects of OSA and hypertension.

FC Abnormality and Nocturnal Intermittent Hypoxia, Sleep Disturbance in OSA

In this study, after adjusting the confounding factors of age, education, BMI, smoking, and alcohol drinking index, we found that the FC between the right hippocampus and the anterior cingulate gyrus was positively correlated with AHI and ODI and negatively correlated with mean SaO_2 among the OSA group, which indicated that chronic intermittent hypoxemia

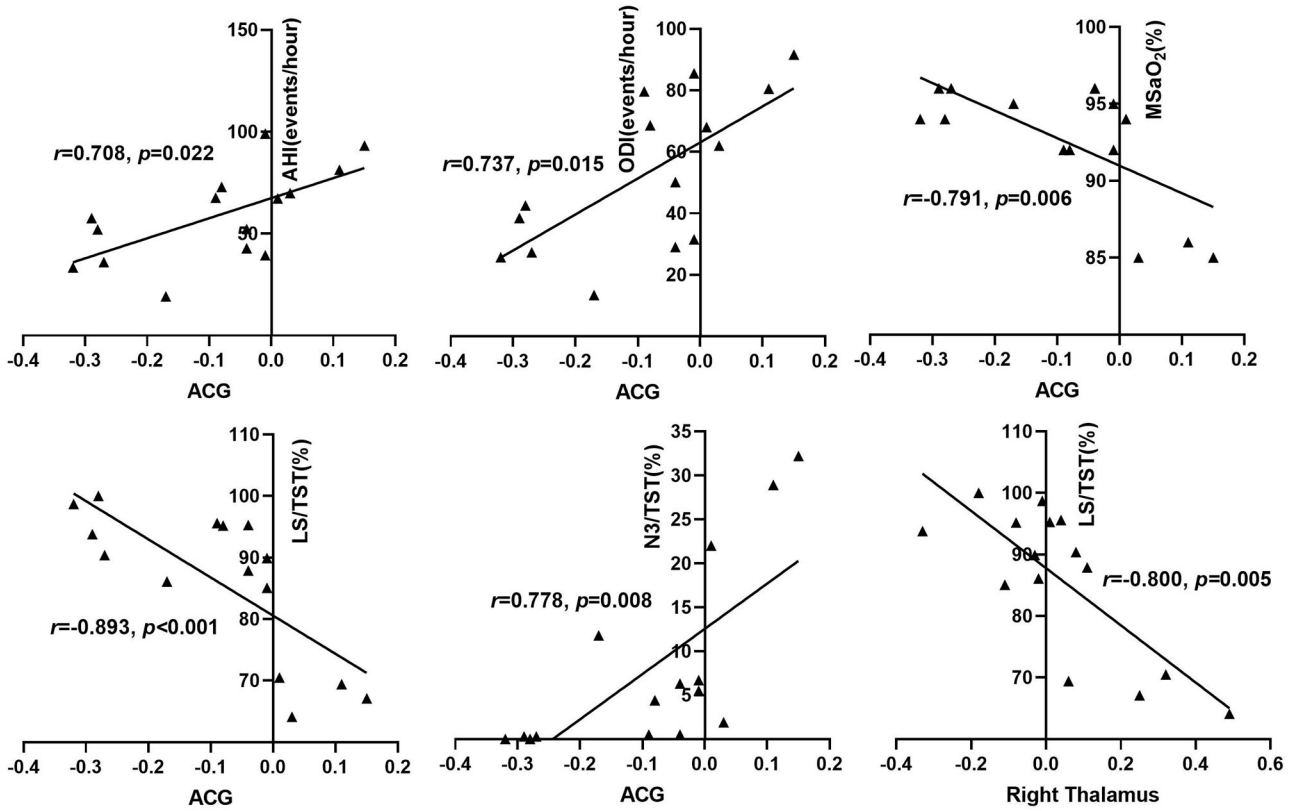

FIGURE 3 | Correlation between reduced right hippocampus FC and sleep respiratory parameters among OSA patients. AHI, apnea-hypopnea index; ODI, oxygen desaturation index; MSaO2, mean oxygen saturation; TST, total sleep time; N3, stage 3 sleep; LS, light sleep; ACG, anterior cingulated gyrus.

TABLE 6 | Correlation between bilateral hippocampal functional connectivity and neurocognitive function among obstructive sleep apnea patients.

***n* = 15**		**HVLT-R**	**Symbol coding**	**CPT-2D**	**CPT-3D**	**SWCT**
RIL	*r* =	0.214	−0.540	−0.261	0.381	0.688
	p =	0.552	0.107	0.466	0.277	0.028*
RMTG	*r* =	−0.661	0.037	−0.003	−0.619	0.120
	p =	0.037*	0.919	0.993	0.056	0.742
RT	*r* =	0.858	−0.372	−0.408	0.269	−0.255
	p =	0.002**	0.290	0.242	0.452	0.476
RSTG	*r* =	0.573	−0.642	−0.351	0.358	0.204
	p =	0.084	0.045*	0.319	0.309	0.571
LAG	*r* =	0.509	−0.638	−0.042	0.104	0.030
	p =	0.133	0.047*	0.909	0.775	0.935
ACG	*r* =	0.510	−0.753	−0.252	0.053	0.059
	p =	0.132	0.012*	0.482	0.884	0.871
LACL	*r* =	0.757	−0.206	−0.351	0.801	0.068
	p =	0.011**	0.567	0.321	0.005**	0.852

*The data were analyzed by the method of partial correlation analysis with adjustment of the confounding factors of age, education, body mass index, smoking index, and drinking index. HVLT-R, Hopkins Verbal Learning Test—Revised; CPT, Continuous Performance Test; SCWT, Stroop Color–Word Test; RIL, right insula lobe; RT, right thalamus; ACG, anterior cingulated gyrus; LACL, left anterior cerebellar lobe; RT, right thalamus; RMTG, right middle temporal gyrus; RSTG, right superior temporal gyrus; LAG, left angular gyrus. *$p < 0.05$ and **$p < 0.01$.*

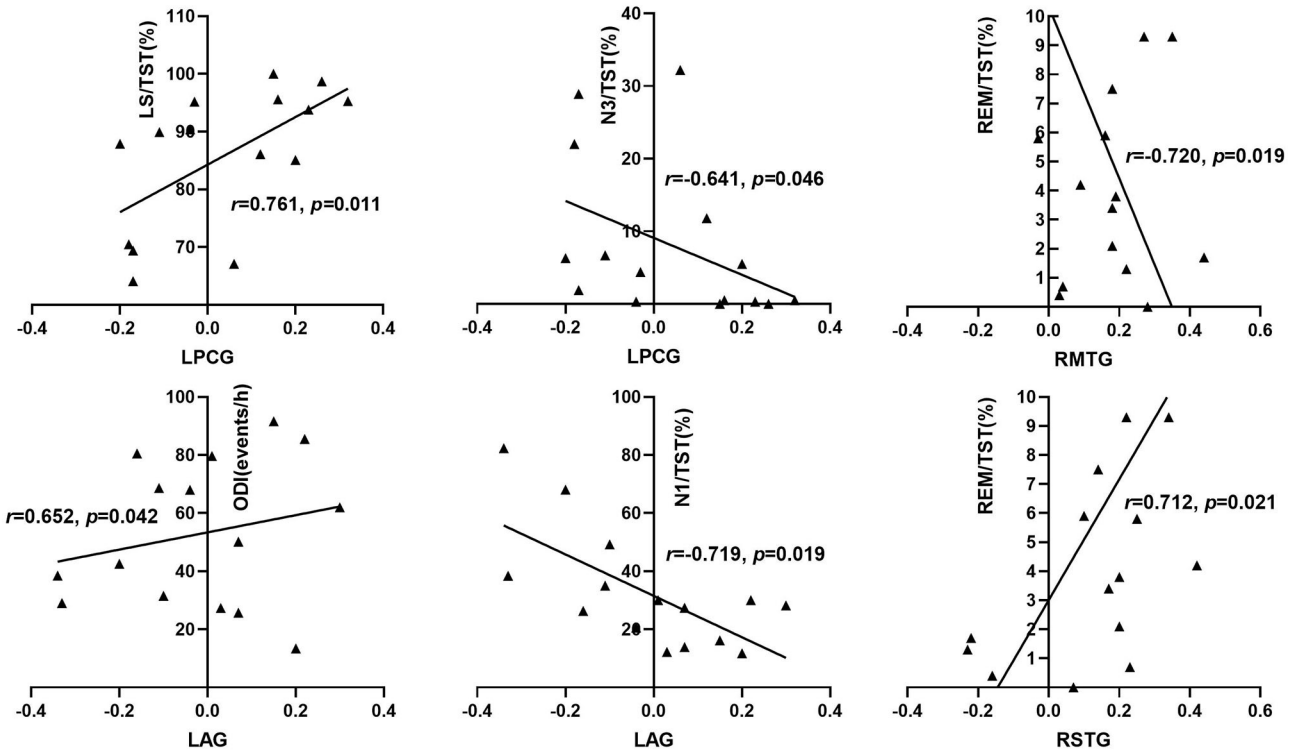

FIGURE 4 | Correlation between increased right hippocampus FC and sleep respiratory parameters among OSA patients. ODI, oxygen desaturation index; ESS, Epworth sleep scale; TST, total sleep time; REM, rapid eye movement; N1, stage 1 sleep; N3, stage 3 sleep; LS, light sleep; LPCG, left posterior cingulate gyrus; RMTG, right middle temporal gyrus; RSTG, right superior temporal gyrus; LAG: left angular gyrus.

may play a role in the abnormality of FC within these two brain regions. The disrupted FC between bilateral hippocampus and anterior cingulate gyrus was also observed in patients with AD (49). Zhang et al. found that the FC within the anterior DMN network was related to AHI and considered that anterior DMN dysfunction in OSA patients may be associated with intermittent hypoxia and enhanced FC in the posterior DMN may be responsible for functional compensation in the brain (23). The hippocampus is susceptible to attacks of hypoxia and oxidative stress that are critical pathological features of OSA. The coordinated interaction between the hippocampus and the anterior cingulate gyrus is mainly involved in information processing, memory consolidation, and formation of recent and long-term memory (50, 51), which suggests that a possible correlation may exist between the abnormal hippocampus FC with the anterior cingulate and neurocognitive dysfunction in OSA. In addition, we found that the changes of hippocampus FC in other brain regions were also closely associated with the disturbance of sleep architecture of patients with OSA. Disturbed sleep structure is a prominent feature of patients with OSA, mainly manifested as typically increased light sleep (including N1 and N2 stage sleep) and reduced/absent deep sleep (including N3 and REM sleep) (52). The features of sleep structure of OSA patients in this study were consistent with the previous findings, which presented with increased light sleep stage and decreased N3 and REM sleep (**Table 1**). The disruption of sleep rhythm could result in disrupted synaptic homeostasis, metabolic dysfunction, excessive oxidative stress, neuro-inflammation, and decreased clearance of brain's metabolites and thus accelerates the pathogenesis of neurodegenerative disorders (53).

In the current study, the right hippocampus FC with the right thalamus or anterior cingulate gyrus both showed a negative correlation with light sleep %TST, while the right hippocampus FC with the left posterior cingulate gyrus showed a positive correlation with light sleep %TST. As we have described before, the right hippocampus revealed a reduced FC with both the right thalamus and the anterior cingulate gyrus but an enhanced FC with the posterior cingulate gyrus. It means that the elevated proportion of light sleep %TST was related to the reduced right hippocampus FC with the right thalamus and the anterior cingulate, a part of the anterior DMN, and the functional compensation between the right hippocampus and the posterior cingulate, a part of the posterior DMN.

It is widely acknowledged that N3 sleep and REM sleep have a fundamental part in the physiological functions of the hippocampus such as memory consolidation, memory re-activation, synaptic homeostasis, *etc*. (54) In this study, the proportion of N3 sleep %TST was positively correlated with the FC between the right hippocampus and the anterior cingulate gyrus and negatively correlated with the FC between the right

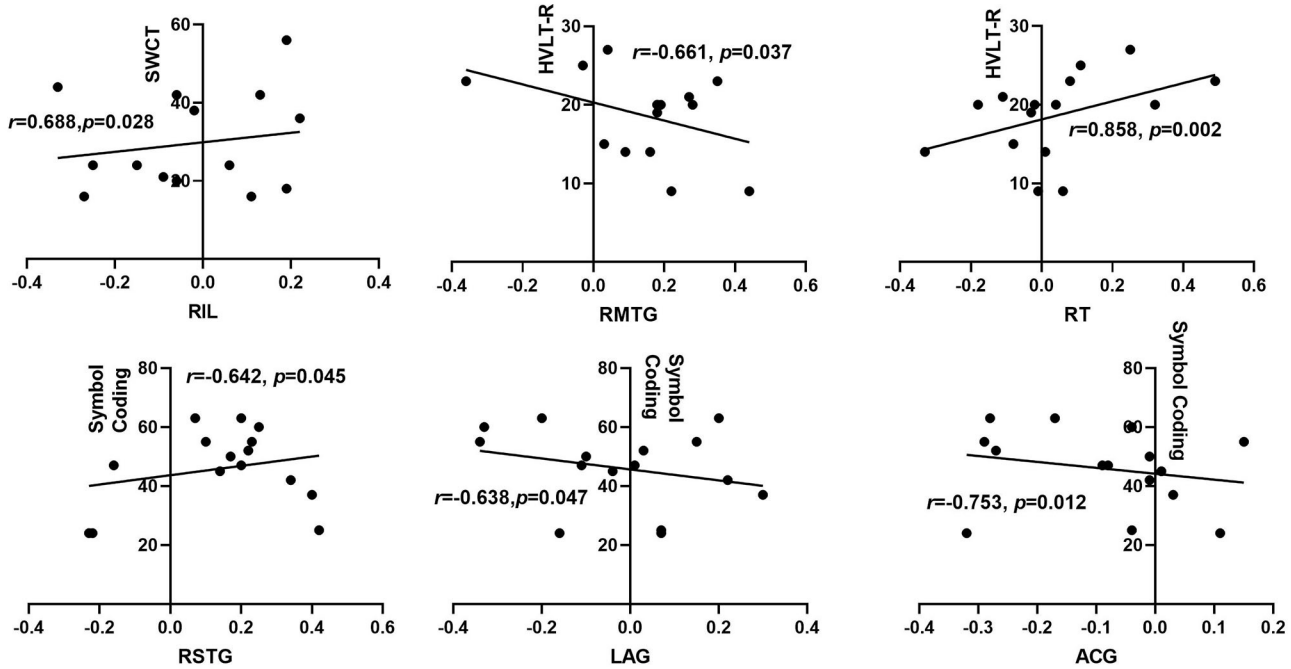

FIGURE 5 | Correlation between right hippocampal FC and neurocognitive function among OSA patients. HVLT-R, Hopkins Verbal Learning Test-Revised; SCWT, Stroop color-word test; RIL, right insula lobe; RT, right thalamus; ACG, anterior cingulated gyrus; RT, right thalamus; RMTG, right middle temporal gyrus; RSTG, right superior temporal gyrus; LAG, left angular gyrus.

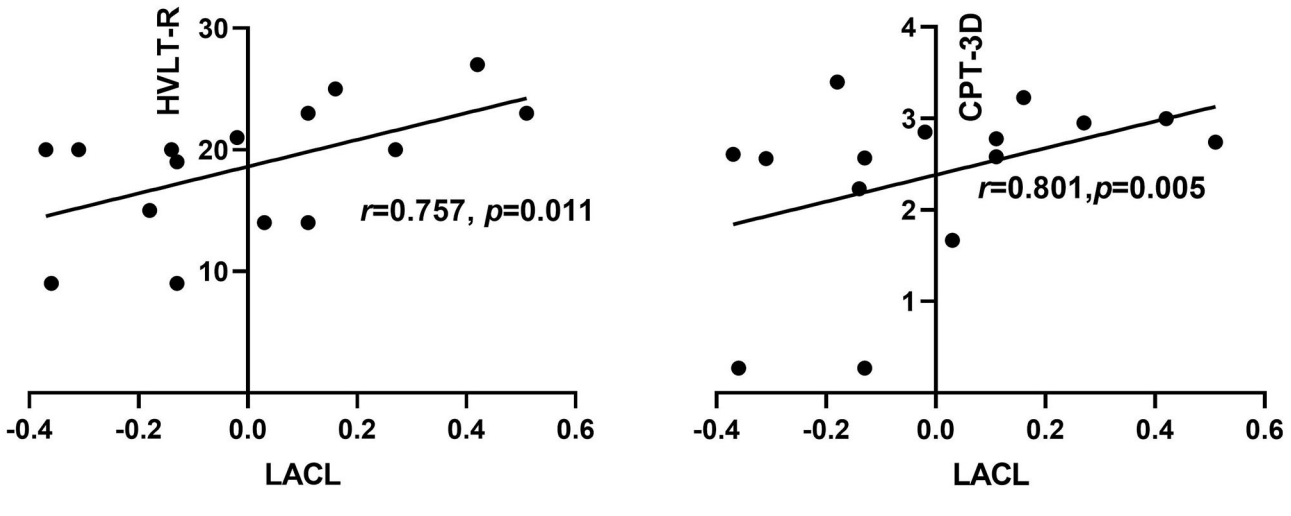

FIGURE 6 | Correlation between left hippocampal FC and neurocognitive function among OSA patients. HVLT-R, Hopkins Verbal Learning Test- Revised; CPT, Continuous Performance Test; LACL, left anterior cerebellar lobe.

hippocampus and the left posterior cingulate gyrus in the OSA group. These findings were coincident with the results of the FC changes between the right hippocampus and the cingulate gyrus, indicating that reduced N3 sleep was involved in DMN–hippocampal functional abnormality by disrupting the right hippocampus functional connection with the anterior DMN and enhancing the functional connection within the posterior DMN. N3 sleep, also known as slow wave sleep, plays a key role in declarative memory consolidation, re-activation of memory traces, clearing metabolites of the brain, orchestrating synaptic downscaling, and regulation of attention (55). The cingulate–hippocampal functional connection is involved in memory, learning, information processing, and emotion activity (56). We consider that disrupted N3 sleep may have an impact

on the alteration of the right hippocampus FC with cingulate gyrus, which is likely to be a part of the pathophysiological mechanism for the pathogenesis of neurocognitive impairment in OSA.

With regards to the role of REM sleep in the changes of hippocampus FC, we found that the FC between the right hippocampus and the right middle temporal gyrus was negatively related to the percentage of REM sleep %TST in patients with OSA, while the FC between the right hippocampal and the right superior temporal gyrus was positively related to the percentage of REM sleep %TST. It has been mentioned in the previous paragraph that increased functional connection presented between the right hippocampus and the right middle and superior temporal gyrus in the OSA group. This means that, when the proportion of REM sleep in TST was high, compensation of FC between the right hippocampus and the right superior temporal gyrus would be strong, and when the percentage of REM sleep in TST was less, the compensational FC between the right hippocampus and the right middle temporal gyrus would become strong. This indicates that the proportion of REM sleep may have no effect on the overall functional connection strength between the right hippocampus and the right temporal lobe. REM sleep is mainly responsible for procedural memories consolidation and emotional memories consolidation (57), while the hippocampal–temporal functional connection is mainly involved in declarative memory and recognition memory such as recollection and familarity (58). The above-mentioned differences between REM sleep and hippocampal–temporal functional interaction in roles in memory reasonably explain the findings involving the relationship between REM sleep and increased FC between the right hippocampus and the right temporal gyrus in patients with OSA. The mechanism resulting in the enhanced hippocampal–temporal gyrus and its value in neurocognitive function in OSA remain to be further explored.

In summary, the above findings manifested that intermittent hypoxemia and sleep fragmentation may be involved in the abnormality of hippocampal FC with other regions. We speculated that FC damage between the right hippocampus and anterior cingulate gyrus may result from joint effects of nocturnal intermittent hypoxia and disturbance of sleep architecture. Meanwhile, reduced left hippocampus FC and the compensation of FC between the right hippocampus and the temporal gyrus were more likely to result from disruption of REM sleep, which further provides a new insight to explore the potential mechanism of neurocognitive impairment in OSA.

Hippocampla FC Abnormality and Neurocognitive Dysfunction in OSA

In this study, we found that sleep parameters including total sleep time, sleep efficiency, light sleep, and REM sleep were closely related to the performance of information processing speed and memory in the OSA group (**Table 2**). The scores of category fluency were negatively correlated with total sleep time and sleep efficiency, indicating that sleep promoted cognitive dysfunction in patients with OSA. We consider that a large amount of sleep fragmentation and low-quality sleep cause the above-mentioned phenomenon in OSA. The proportion of light sleep in total sleep time was negatively correlated with the scores of memory tests, and the proportion of REM sleep in TST was positively associated with the scores of information processing speed test, indicating that disrupted sleep structure was involved in neurocognitive dysfunction in patients with OSA. In the previous section, it has been demonstrated that disrupted sleep architecture was involved in the abnormality of hippocampus FC with other brain regions in OSA. Combined with the above-mentioned findings, we speculated that abnormal hippocampus FC was involved in the pathogenesis and development of neurocognitive impairment in patients with OSA.

As expected, we found that the FC strength between the right hippocampus and the right insular lobe and thalamus were positively correlated with the performance of memory and executive function in the OSA group, in which the FC between the right hippocampus and the right insular lobe and thalamus were reduced compared with that in the HCs group. It indicates that the functional deficiency in these brain regions probably participates in the development of the impairment of memory and executive function in OSA. Meanwhile, the FC strength between the right hippocampus and the right superior temporal gyrus and left angular gyrus was negatively correlated with the performance of information processing speed, and the FC strength between the right hippocampus and the right middle temporal gyrus was also negatively correlated with the performance of memory in patients with OSA, in which the FC within these brain regions was enhanced compared with that of HCs. This suggests that an increased FC between the right hippocampus and other brain regions has not shown beneficial effects on alleviating neurocognitive impairment. The possible explanation for this finding is that the functional compensation between the right hippocampus and the left angular gyrus and right temporal gurus is incomplete to be able to prevent neurocognitive decline in patients with OSA. On the other hand, the FC abnormality within these brain areas probably promoted the neurocognitive dysfunction of OSA.

Another novel finding in our study is that we found that the left hippocampal FC with the left anterior cerebellum was significantly declined in patients with OSA compared with those in HCs. The FC between the left hippocampal FC and the left anterior cerebellum lobe was positively correlated with the percentage of REM sleep %TST, demonstrating that REM sleep might play a role in regulating the hippocampus functional interaction with the cerebellum. Besides these, the FC between the left hippocampus and the left anterior cerebellum showed a positive relationship with the performance of memory and attention test. To our current knowledge, this is the first time to discover that abnormal FC between the hippocampus and the cerebellum might be involved in neurocognitive impairment in patients with OSA. The hippocampal–cerebellar interaction has been demonstrated to play an important role in procedural memory, episodic memory, emotional memory, and information processing (spatial and temporal processing) (59). Previous studies have demonstrated that, compared with HCs, the gray matter volume and the density of cerebellum in patients with

OSA were significantly reduced, and it was considered that intermittent hypoxia and sleep fragmentation may result in cerebellar structural changes (13, 60). We speculated that the disruption of REM sleep may, to some extent, cause the functional disconnection between the hippocampus and the cerebellum and contribute to declined memory and attention in the long term in OSA. The cerebellar–hippocampal functional disconnection may be a promising and novel mechanism of neurocognitive impairment of OSA.

All of above-mentioned findings suggest that FC abnormalities between the hippocampus and other brain regions may contribute to declined attention, executive function, and memory in patients with OSA. The hippocampus is extremely sensitive to hypoxia and oxidative stress. The typical pathophysiological alterations in OSA, including intermittent hypoxia/re-oxygenation, hypercapnia, changes of cerebral blood perfusion, and sleep fragmentation, could cause an accumulation of harmful metabolites, oxidative stress products, and neuro-inflammation cytokines (61), further resulting in functional disruption, pathological activities, and even structural changes in the hippocampus, ultimately leading to neurocognitive dysfunction.

However, there are some limitations of this study: Firstly, the sample size of this study is small, which may restrain the representativeness of the above-mentioned results. In order to minimize the effect of head movement on FC, we used a stricter inclusion criterion for head movement to obtain more reliable results, which led to the fact that a small number of OSA patients were included in this study. Secondly, this study was a clinical observational study with correlation analysis which cannot conclude an exact mechanism for neurocognitive impairment. Thirdly, patients with mild OSA were excluded from this study, which might have prevented us from generalizing the results to the whole population of OSA. Fourthly, moderate–severe OSA patients with a complication of hypertension or nocturnal hypertension were not excluded in this study. Hypertension is extremely common in OSA, with a high prevalence of up to 70%. Finally, due to a much lower prevalence in women, a much lower population of female patients was involved in the current study, which might weaken the generalizability of the findings to the whole population.

CONCLUSION

Abnormal FC and adaptive compensation were demonstrated between the hippocampus and the cerebrum and cerebellum in patients with moderate to severe OSA. The right hippocampus mainly showed an altered FC with the cerebral cortex, subcortex, or DMN, while the left hippocampus mainly presented a changed FC with the left anterior cerebellar lobe. Moreover, this extensive abnormality of the right hippocampal FC may result from the joint effect of intermittent nocturnal hypoxia and sleep fragmentation, and the reduced FC between the left hippocampus and the anterior cerebellar lobe was more likely caused by a disturbed sleep architecture. More importantly, the abnormality of hippocampal network FC was closely related to neurocognitive impairment of OSA, including the cognitive fields of memory, attention, and executive function, which indicated a promising insight to explore the potential imaging biomarker and pathophysiologic mechanism of neurocognitive dysfunction of OSA.

DATA AVAILABILITY STATEMENT

The original contributions generated for the study are included in the article, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of The Second Xiangya Hospital, Central South University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

LZ designed, conducted the study, and was a major contributor in writing the manuscript. GL contributed to data analysis. RO supervised, designed the study, and revised the manuscript. The rest of the co-authors contributed to data collection. All authors contributed to the article and approved the submitted version.

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Abnormal Brain Network Topology During Non–rapid Eye Movement Sleep and Its Correlation With Cognitive Behavioral Abnormalities in Narcolepsy Type 1

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Objective: Simultaneous electroencephalography (EEG) and functional magnetic resonance imaging (fMRI) were applied to investigate the abnormalities in the topological characteristics of functional brain networks during non-rapid eye movement(NREM)sleep. And we investigated its relationship with cognitive abnormalities in patients with narcolepsy type 1 (NT1) disorder in the current study.

Methods: The Beijing version of the Montreal Cognitive Assessment (MoCA-BJ) and EEG-fMRI were applied in 25 patients with NT1 and 25 age-matched healthy controls. All subjects participated in a nocturnal video polysomnography(PSG)study, and total sleep time (TST), percentage of TST (%TST) for each sleep stage and arousal index were calculated. The Epworth Sleepiness Score (ESS) was used to measure the degree of daytime sleepiness. The EEG-fMRI study was performed simultaneously using a 3T MRI system and a 32-channel MRI-compatible EEG system during sleep. Visual scoring of EEG data was used for sleep staging. Cognitive function was assessed for all subjects using the MoCA-BJ. The fMRI data were applied to establish a whole-brain functional connectivity network for all subjects, and the topological characteristics of the whole-brain functional network were analyzed using a graph-theoretic approach. The topological parameters were compared between groups. Lastly, the correlation between topological parameters and the assessment scale using Montreal Cognition was analyzed.

Results: The MoCA-BJ scores were lower in patients with NT1 than in normal controls. Whole-brain global efficiency during stage N2 sleep in patients with NT1 displayed significantly lower small-world properties than in normal controls. Whole-brain functional network global efficiency in patients with NT1 was significantly correlated with MoCA-BJ scores.

Conclusion: The global efficiency of the functional brain network during stage N2 sleep in patients with NT1 and the correspondingly reduced small-world attributes were associated with cognitive impairment.

Keywords: cognitive dysfunction, graph theory analysis, type 1 narcolepsy disorder, functional connectivity, sleep, Montreal cognitive assessment Beijing edition

INTRODUCTION

NT1 is a chronic neurological disorder characterized by irresistible daytime sleepiness, cataplexy, sleep hallucinations, sleep paralysis, and nocturnal sleep disturbances (1). Patients with NT1 typically show cataplexy and a dramatic reduction in the cerebrospinal fluid concentration of the neuropeptide orexin A (hypocretin 1), which results from extensive loss of the orexin-producing neurons in the hypothalamus (2).

Several current psychiatric studies on patients with NT1 disorder have suggested that these patients have multiple cognitive deficits, including abnormal emotional learning (3), deficits in subjective perception of attention (4), lack of perseverance and selectivity in decision making (5), and deficits in the ability to activate attention and arousal-related areas (6).

To explore the neural mechanisms underlying NT1 disorder combined with cognitive impairment, several neuroimaging studies have been conducted in the last decade. Among them, structural imaging studies have revealed significant abnormalities in the bilateral hippocampus, amygdala (7–10) and left marginal superior gyrus (11) gray matter, as well as in the bilateral cerebellar hemispheres, bilateral thalamus, hypothalamus, corpus callosum, and left anterior medial temporal white matter, in patients with NT1 disorder (12–14). In contrast, functional (fMRI) studies based on intercerebral connectivity have revealed impairment in the executive attention network (15) and abnormal functional connectivity (16, 17) in patients with NT1 disorder, and the presence of an alternative neural circuit that controls emotional responses to emotional challenges (18). Patients with NT1 disorder have a cortical neural network involved in the processing of rewards and emotions (19) with lower thresholds (20) or overactivation, associated with cataplexy (21, 22). Other studies have reported that alterations in brain connectivity and topology of some brain regions in patients with NT1 disorder are associated with lethargy, depression, and impulsive behavior (23).

However, the results of these studies are not entirely consistent, and very little work has been performed to assess the overall topological properties of whole-brain neural networks in patients with NT1 disorder. In addition, the above studies, especially those employing fMRI, were performed during the awake state. fMRI studies looking at functional brain network properties in patients with NT1 disorder in the sleep state, however, have not been reported.

The simultaneous acquisition of EEG and fMRI data provides a noninvasive method of investigating brain function during sleep (24) EEG data can be used to determine the stages of sleep, while fMRI analysis data can provide insight into the neural activity at different stages (25).

The aim of this study was to analyze the characteristic changes in brain functional connectivity during sleep, in patients with NT1 disorder, using noninvasive simultaneous EEG, fMRI and its relationship with neurobehavioral abnormalities.

MATERIALS AND METHODS

Objectives

This study recruited 36 right-handed patients (male: female ratio, 21:15; age, 10.0–27.0 years) with NT1 disorder diagnosed according to the International Classification of Sleep Disorders (ICSD)-3 (26), from the Sleep Medicine Center of Shengjing Hospital, China Medical University. Another 33 right-handed healthy controls (male: female ratio, 19:14; age, 11.7–28.6 years) were recruited from the community. We excluded 19 participants: 4 patients with NT1 disorder and 3 volunteers withdrew from the PSG examination, and 7 patients and 5 volunteers failed to fall asleep, were unable to enter the N2 phase of sleep, or had excessive head motion resulted in failure to obtain fMRI data containing the complete sleep stage (including stage N1 and stage N2). Therefore, we analyzed data from 25 patients with NT1 (age 22.4 $\pm$ 6.9 years, 64% male) and 25 healthy controls (age 22.5 $\pm$ 3.8 years, 60% male). This study was approved by the ethics committee of Shengjing Hospital, China Medical University Medical College. In accordance with the Declaration of Helsinki, all participants had signed an informed consent form to participate in the experiment prior to the study.

Admission criteria for patients with NT1 disorder were as follows. According to the International Classification of Sleep Disorders for Episodic Sleep Disorders, a sleep specialist delivered a diagnosis based on 3 months of excessive daytime sleep and a clear clinical history of cataplexy. The final diagnosis was made by nocturnal PSG and the Multiple Sleep Latency Test (MSLT); the severity of EDS was measured using the ESS in patients with episodic sleeping disorder and healthy controls. In the ESS measure, participants were asked to describe, on a scale from 0 to 3, how likely they were to fall asleep in eight different situations, and if the total score was $\geq$ 10, a diagnosis of excessive daytime sleepiness was made (26).

Exclusion criteria for patients with NT1 disorder and healthy volunteers were as follows: subjects with other sleep disorders; current or previous severe physical or neurological disorders; routine MRI scan findings of brain abnormalities (tumors, hemorrhages, infarct foci); history of severe psychiatric or neurological disorders in the immediate family; current or previous psychiatric disorders such as depression, anxiety, or

substance abuse; congenital genetic disorders; and presence of contraindications to MRI examination.

All subjects including patients with NT1 disorder and healthy volunteers, underwent a comprehensive neurological examination prior to the MRI scan to rule out peripheral and central nervous system disorders. The full procedure of the experiment, its purpose, clinical significance and precautions were explained to each subject before the start of scanning, and all patients with NT1 disorder and healthy volunteers who participated in this study signed an informed consent form.

Epworth Sleepiness Score (ESS)

The ESS was used to measure the degree of daytime sleepiness in patients with NT1 disorder and healthy controls. In the ESS measure, participants were asked to describe, on a scale from 0 to 3, how likely they were to fall asleep in eight different situations, and if the total score was $\geq$ 10, a diagnosis of excessive daytime sleepiness was made (26).

Polysomnography (PSG) and Assessment of Excessive Daytime Sleepiness

Prior to study participation, all patients were asked to abstain from drinking coffee or alcoholic beverages for one day. All night polysomnograms were recorded on a respiratory electronic series physiological monitoring system (Alice 6, Philips, Murrysville, FL, USA) the day before the MRI examination. PSG was recorded from 22:00 to 06:00, including EEG, electro-oculogram, electrocardiogram, etc. Standard EEG (frontal, central, occipital EEG: F4/M1, C4/M1, O2/M1, backup F3/M2, C3/M2, O1/M2), mandibular EMG (3 mandibular electrodes and EMG in the middle of the right tibialis anterior muscle), EOG (EOG located in the cornea and retina), and ECG were recorded according to the American Academy of Sleep Medicine guidelines. Oral and nasal airflow, snoring, chest and abdominal breathing, oxygen saturation and position, as well as total sleep time, sleep latency, sleep efficiency, arousal, and breathing events, were all recorded. According to the American Academy of Sleep Medicine Manual, a decrease in airflow of $\geq$ 90% and lasting for at least 10 s is defined as obstructive apnea and is associated with sustained respiratory effort; a decrease in airflow of $\geq$ 30% and lasting for at least 10 s is defined as hypopnea with 4% or higher oxygen desaturation (27). The apnea hypopnea index was calculated from the mean of the total number of apnea and hypopnea events experienced per hour of sleep. All patients completed a clinical MSLT the day after nocturnal PSG. With reference to the modifications approved by the American Academy of Sleep Medicine Task Force (28), naps were scheduled every 2 h starting 2 h after first awakening in the morning. If the patient did not sleep within 20 min, the NAP test was terminated, and the sleep latency was recorded for 20 min. If sleep occurred within 20 min, the onset time was defined as the time from lights out to the first sleep period (including stage 1). To assess the presence of rapid eye movement(REM)sleep, monitoring was continued for $\geq$ 15 min after sleep onset. If REM sleep was present, the latency of REM sleep was also recorded. The mean sleep latency and REM sleep latency of the five NAPs in the MSLT, were then calculated.

PSG data and EEG data were analyzed by an experienced technologist who had no knowledge of the clinical or demographic status of the participants. The results of the analysis were then verified by a physician. According to the American Academy of Sleep Medicine (AASM) criteria, participant's sleep was scored according to the staging of sleep, and related events, such as cortical arousal and wakefulness. The TST and % TST (NREM, including stages N1-N3 and REM sleep) for each sleep stage were calculated, along with the arousal index (A index, indicating observed sleep disruptions).

Montreal Cognitive Assessment Beijing (MoCA-BJ)

All cognitive function tests were assessed using the MoCA-BJ (29). The test included cognitive domains such as visual-spatial and executive ability (5 points), naming (3 points), attention and computation (6 points), language (3 points), abstraction (2 points), delayed recall (5 points), and orientation (6 points), with

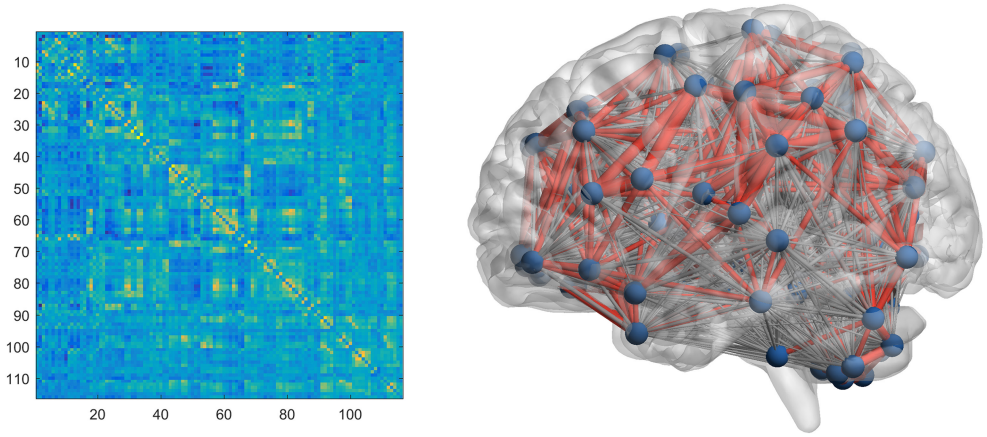

FIGURE 1 | Construction of brain network in the participants.

a total score of 30 points and a duration of 10 min. A score of < 26 was considered cognitive impairment.

The Stanford Sleepiness Scale (SSS) (30) was used to asses sleepiness during the MoCA-BJ testing session. All participants filled in the scale before and after the testing session. The patients with NT1 had a mean score of 1.7 (SD = 0.9) before and 1.9 (SD = 1.1) after the test session. The healthy controls had a mean score of 1.1 (SD = 0.5) before and 1.4 (SD = 0.7) after the testing session.

EEG-MRI Scan

Simultaneous EEG-fMRdeepI data were acquired within one week after multi-conductor EEG monitoring. All images were acquired using a Philips 3.0T superconducting MRI scanner (Philips Medical Systems, Best, Netherlands). The head scan coil was a 32-channel RF magnetic head coil and a net amplifier 300, with 32-channel electrode caps. The subject was placed in a comfortable supine position, flat on the MRI bed, wearing noise-reducing earplugs, and the subject's head was immobilized with foam padding, to reduce head movement. And the amount of impaired sleep in this study was within the same range as in previous studies (31). To exclude any obvious organic brain lesions, all patients with episodic sleeping sickness and healthy controls underwent a routine axial T2WI scan. The angular line was used as the baseline for the scan, and an experienced radiologist read the films on site at the end of the scan. The patient was informed of the scan time and instructed not to resist sleep during the entire EEG-fMRI scan. The subject was asked to keep the head as still as possible during the scan. When the subject signals showed that they could no longer sleep in the scanner, the scan was terminated.

The fMRI data were acquired using a gradient-echo plane echo sequence, with time repetition (TR) = 2 s, time echo (TE) = 30 ms, spatial resolution = 3.5 × 3.5 × 4 mm^3, matrix size = 64 × 64, Field of View (FOV) = 220 × 220 mm, flip angle (FA) = 90°, sensitivity encoding factor (SEF) = 2, number of layers scanned. (number of slices) = 31, and layer thickness = 0 mm. The number of consecutive volume runs was collected 1,200 times, and an additional virtual scan (8 s in duration) was applied at the beginning of the fMRI scan, to stabilize the magnetic field and eliminate chemical displacement artifacts. Thus, the duration of the fMRI scan for each segment was 40 min and 8 s.

EEG data acquisition was synchronized with the MRI scanner clock. Thirty MRI-compatible scalp electrodes were placed in reference to the International 10–20 system; additional channels were used for ECG signal recording. The impedance of the electrodes was kept below 20 kΩ during signal acquisition. The sampling rate of the EEG data was 10 kHz. The frequency range of the hardware filter was 0.016 to 250 Hz.

EEG Pretreatment and Sleep Staging

Net Stations 5.4 package was used to remove gradient and pulse artifacts from the EEG data. Pre-processed EEG data were sleep staged according to AASM criteria without overlap, with each epoch lasting 30 consecutive seconds, and then compared to a standard sleep montage by a qualified neurophysiologist.

Functional Magnetic Resonance Imaging Preprocessing

The fMRI data were pre-processed on MATLAB version R2016a platform using the SPM12 package (Wellcome Trust Center for Neuroimaging at UCL, London, UK). The fMRI data from the different layers were first temporally corrected. The images were then re-aligned to the first layer of images for head motion correction and to estimate the head motion parameters (translation and rotation) for each direction. The resulting image space was then normalized to the Montreal Neurological Institute (MNI) labeled space with a resampled voxel of 3 × 3 × 3 mm. The half-height full width (FWHM) of the Gaussian kernel was set to 6 mm, and the normalized images were spatially smoothed; the spatially smoothed images were time-filtered (0.01-0.15 Hz). Finally, head movement parameters, ventricular signals, and brain white matter signals were regressed using the Data Processing and Analysis of Brain Imaging (DPABI) software package (32) (http://rfmri.org/dpabi). The pre-processed data were then divided into epochs lasting 30 consecutive seconds. Each epoch was assigned a particular sleep stage determined by simultaneously acquired EEG data. The minimum acceptable length of fMRI data for each sleep stage with a subject was 8 epochs (4 min).

TABLE 1 | Demographics data of study participants.

	Patients ($n = 25$)	Healthy controls ($n = 25$)	*Chi-square*, *t* or *Z* value	*P* value
Age	22.4 (6.9)	22.5(3.8)	0.063	0.95
Sex (% male)	16 (64%)	15 (60%)	0.085	0.77
MoCA-BJ scores	25 (25–26)	30 (0–0)	25	<0.0001#,*
Disease duration (years)	7.1 (3.9)	-		

Data were presented as median (IQR), frequency (%), or mean (SD).
#P value were corrected using Holm-Bonferroni method.
**P < 0.05.*

TABLE 2 | PSG data in NT1 participants and healthy controls.

	Patients ($n = 25$)	Control ($n = 25$)	*Chi-square*, *t* or *Z* value	*P* value
TST	7.0 (0.8)	7.2 (0.9)	0.831	0.41
Sleep stage (% TST)				
N1	12.7 (2.6)	6.7(2.4)	8.479	< 0.0001#,*
N2	51.1 (4.8)	46.4 (4.9)	3.426	0.001
N3	22.8(8.2)	33.4 (7.9)	4.655	< 0.0001#,*
REM	13.4 (7.3)	13.5 (6.1)	0.053	0.96#
A index	21.5 (7.9)	4.0(2.3)	10.630	< 0.0001#,*

Data were presented as median (IQR), frequency (%), or mean (SD).
#P value were corrected using Holm-Bonferroni method.
**P < 0.05.*

Brain Network Structure and Graph Theory Analysis

A graph-theoretical network analysis toolbox, Gretna, was used to target the completed preprocessed fMRI data in order to construct functional brain networks in each for all epochs (33). To this end, the entire brain was divided into 90 cortical and subcortical regions based on automated anatomical label (AAL) markers (34), and the average time series of the 90 regions was extracted. A Pearson's correlation coefficient for each pair of regions was calculated for the average time series of all 90 regions, and the data were transformed into z-values, considered to have a normal distribution using Fisher's z-transform. A positively binary vector less connected generic network was then constructed based on the chosen threshold range of the correlation matrix. After conversion, the z-scored correlation coefficients assigned to the same sleep stage were averaged for each participant (**Figure 1**).

Graph theory analysis was used to assess the topological and organizational properties of the entire brain. Topological measures can be divided into global efficiency (Eglob) and local efficiency (Eloc) (35) and small-world network parameters (36) (**Supplement 1**). Sparsity was used as a correlation measure for the correlation coefficient threshold range, defined as the number of available edges in the graph divided by the maximum number of possible edges. According to previous research (35), we set both the sparsity and Pearson correlation thresholds of the function network in the range of 0.05 to 0.5 (in 0.05 steps) to obtain more efficient ubiquitous networks, when compared to random networks with the lowest number of artificial edges.

Data Analysis

This study used IBM SPSS software (version 17, IBM Inc., Armonk, NY, USA) to analyze demographic, cognitive data, brain function network data, and PSG data. The Kolmogorov-Smirnov test was used to test the hypothesis of normality for continuous variables. Descriptive statistics were firstly performed. Continuous data with a normal distribution were expressed as mean ± standard deviation (SD), and non-normally distributed data were expressed as the median of the interquartile range. These data include age, and questionnaire score. Comparisons were made between the patient with NT1 disorder group and the healthy control group, using *t*-tests or Mann Whitney *U*-tests based on the normality and homoscedasticity of these variables. A $\chi 2$ or Fisher's exact test was taken to compare sex ratios between the different groups. The demographic characteristics of the subjects who dropped out were also compared with those of the subjects who remained. The *t*-test or Mann-Whitney *U*-test was also used to compare PSG data and topological parameters of the brain functional network during sleep between the two groups. The Holm-Bonferroni method was used to correct for multiple comparisons. Statistical significance for all the above statistical analyses required a two-tailed test with a level < 0.05.

A Spearman's correlation analysis was used to explore the relationship between MoCA and brain network parameters in patients with NT1 disorders. $P < 0.05$ was defined as a significant difference.

RESULTS

This study contains demographic, PSG, MoCA-BJ scale, and imaging data for 25 patients with NT1 disorder and 25 healthy volunteers (50 participants in total).

TABLE 3 | Brain functional network parameters in NT1 participants and healthy controls.

	Patients ($n = 25$)	Healthy controls ($n = 25$)	*Chi-square, t or Z* value	*P* value
aEg	0.24 (0.05)	0.29 (0.06)	3.201	$< 0.007^{\#,*}$
aEloc	0.32(0.03)	0.36(0.07)	2.626	$< 0.015^{\#,*}$
aSigma	0.61(0.08)	0.73 (0.11)	4.927	$< 0.001^{\#,*}$

Data were presented as median (IQR), frequency (%), or mean (SD).
#P value were corrected using Holm-Bonferroni method.
**P < 0.05.*

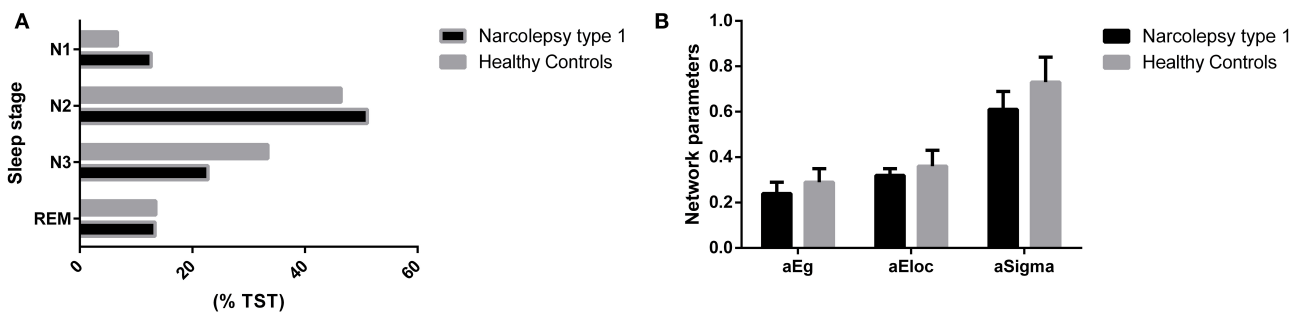

FIGURE 2 | Comparisons of %TST and cerebral topological parameters between groups. **(A)** Compared with the control group, patients with NT1 disorder had significantly higher percentage of N1 and N2 during TST ($t = 8.479$, $P_{corrected} < 0.0001$; $t = 3.426$, $P_{corrected} < 0.001$) and significantly lower %TST during N3 ($t = 4.655$, $P_{corrected} < 0.0001$). **(B)** Compared with the control group, patients with NT1 disorder had significantly lower global efficiency and small-world attributes (Bonferroni correction, $P < 0.05$).

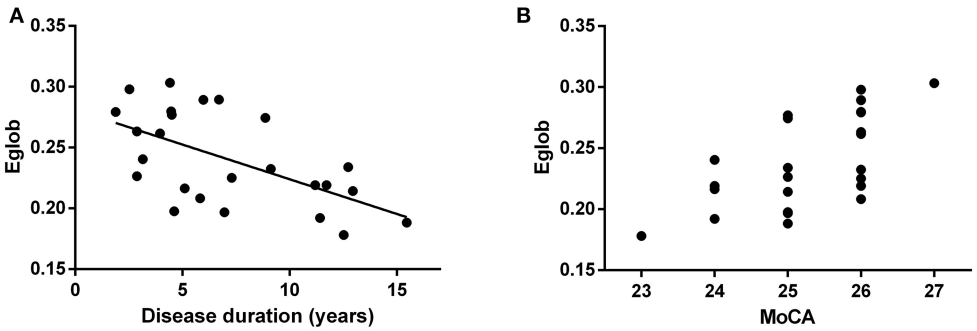

FIGURE 3 | Relationship between the global efficiency of the whole brain functional network, and years of onset, and MoCA score in NT1 participants. **(A)** There was a significant correlation between the global efficiency of the whole brain functional network, in patients with NT1 disorder, and Disease duration in the stage N2 sleep. **(B)** There was a significant correlation between the global efficiency of the whole brain functional network, in patients with NT1 disorder, and MoCA score in the stage N2 sleep.

Table 1 summarizes the demographics of the participants. The two groups were matched for age and sex. In the healthy control group: age 22.5 ± 3.8 years, 60% male; and in the NT1 disorder patient group: age 22.4 ± 6.9 years, 64% male. The MoCA-BJ score was significantly lower in the patient with NT1 disorder group than in the control group ($P < 0.001$).

PSG Data

Patients with NT1 disorder had significantly higher percentage of N1 and N2 during TST ($t = 8.479$, $P_{corrected} < 0.0001$; $t = 3.426$, $P_{corrected} < 0.001$) and significantly lower %TST during N3 ($t = 4.655$, $P_{corrected} < 0.0001$), when compared to controls. The A index was significantly higher in patients with NT1 disorder than in the control group ($t = 10.630$, $P_{corrected} < 0.0001$), but the TST was not significantly different in patients with NT1 disorder, when compared to the control group ($P > 0.05$) (**Table 2**, **Figure 2**).

Brain Network Analysis

Patients with NT1 disorder had significantly lower global efficiency and small-world attributes when compared to healthy controls (Bonferroni correction, $P < 0.05$, **Table 3**). There was a significant correlation between the global efficiency of the whole brain functional network in patients with NT1 disorder, years of onset, and MoCA-BJ score in the stage N2 sleep ($r = -0.589$, $P = 0.002$; $r = 0.632$, $P < 0.001$, respectively) (**Figure 3**).

DISCUSSION

In this study, we found that patients with NT1 disorder have a higher proportion of stage N1 and stage N2 during all night PSG monitoring, which is consistent with previous studies (37). Since it was difficult for participants to reach the stage N3 during MRI scanning due to the noisy environment and only N2 period data of fMRI scanning in this study were available to analyze, this study only analyzed fMRI data from the stage N2 sleep.

Our study found that the global efficiency and small-world properties of the whole brain functional network in patients with NT1 disorder were lower than those in normal controls during stage N2 sleep. Our data suggested that the global efficiency was proportional to the length of onset and correlated with the severity of cognitive impairment. Since the global efficiency of brain networks is mainly influenced by long-distance connections within the network, we speculated that the reduced topological efficiency of brain networks during sleep, may be primarily due to abnormalities in long-distance functional connections. These abnormalities in the topological efficiency of brain networks may be related to the connection anomalies between several brain networks previously found in resting state studies, including altered connectivity between the executive and salience networks (16), reduced inter-node connectivity in the default mode network (38), and reconfiguration of the salience and default mode networks (23). Given previous studies have demonstrated that the brain sensory-motor networks in the resting state, such as the somatomotor network, visual network, auditory network, default mode network, dorsal attention network, abdominal attention network, linguistic network, frontal parietal network, salient network, always exhibited constant neurological activity (39). Thus, the inefficiency of the functional brain networks in the sleep state of NT1 disorder patients is likely to lead to a decrease in information processing ability in the sleep state, which in turn extends to the waking state leading to cognitive impairment.

Although this EEG-fMRI study provides preliminary evidence of abnormalities in brain functional networks in patients with NT1 disorder during sleep, this study has several limitations. First, the number of patients with NT1 disorder was limited. Second, the age distribution of the patients was large. Third, this study only analyzed fMRI data from the stage N2 sleep.

In summary, we found that patients with NT1 disorder have reduced whole-brain functional network efficiency during stage N2 sleep, and that this reduced whole-brain functional network efficiency may be related to their cognitive impairment.

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 Ethical Committee of Shengjing Hospital of China Medical University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

XZ: data curation and writing-original draft preparation. KN: data curation and software. HT: visualization and investigation. YL: writing-reviewing and editing. YZ: validation. BY: conceptualization and methodology. LX: conceptualization, validation, and supervision. 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/fneur.2020.617827/full#supplementary-material

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ORIGINAL RESEARCH
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Altered Percent Amplitude of Fluctuation in Healthy Subjects After 36 h Sleep Deprivation

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Objective: To investigate regional brain activity alteration in healthy subjects in a sleep deprivation (SD) status relative to a rested wakefulness status using a percent amplitude of fluctuation (PerAF) method.

Methods: A total of 20 healthy participants (12 males, 8 females; age, 22.25 ± 1.12 years) were recruited. All participants underwent attention tests and resting-state functional MRI scans during rested wakefulness before SD and after 36 h SD, respectively. The PerAF method was applied to identify SD-related regional brain activity alteration. A ROC curve was conducted to evaluate the ability of the PerAF method in distinguishing different sleep statuses. The relationships between SD-induced brain alterations and attention deficits were determined by Pearson correlation analysis.

Results: SD resulted in a 2.23% decrease in accuracy rate and an 8.82% increase in reaction time. SD was associated with increased PerAF differences in the bilateral visual cortex and bilateral sensorimotor cortex, and was associated with decreased PerAF differences in bilateral dorsolateral prefrontal cortex and bilateral cerebellum posterior lobe. These SD-induced brain alterations exhibited a high discriminatory power of extremely high AUC values (0.993–1) in distinguishing the two statuses. The accuracy rate positively correlated with the bilateral cerebellum posterior lobe, and bilateral dorsolateral prefrontal cortex, and negatively correlated with the bilateral sensorimotor cortex.

Conclusions: Acute SD could lead to an ∼8% attention deficit, which was associated with regional brain activity deficits. The PerAF method might work as a potential sensitivity biomarker for identifying different sleep statuses.

Keywords: sleep deprivation, percent amplitude of fluctuation, receiver operating characteristic, attention network test, visual cortex, cognitive deficit

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INTRODUCTION

Sleep has been increasingly shown to have far more impact on human health than it was previously recognized; however, sleep has been rarely studied by neuroimaging (1). Sleep deprivation (SD) is associated with maladaptive changes in emotion, cognition, immunity (1–9), and even expression of certain genes (10, 11). Although SD has been frequently used

to explore behavioral and functional consequences caused by sleeploss (12, 13), the underlying neurobiological mechanisms of SD remain largely unknown.

During the last decade, modern brain neuroimaging techniques have been extensively used to encourage scholars to investigate the potential pernicious effects on cognitive function and regional brain areas caused by SD (4, 5, 14–18). Resting-state functional MRI (rs-fMRI) has been considered as an applicable and accepted method to address the regional brain activity deficits associated with SD. Regional homogeneity, amplitude of low-frequency fluctuations (ALFF), and fractional ALFF (fALFF) are three important methods of rs-fMRI to address regional brain alterations (5, 19–21), however, these methods could be easily influenced by physiological high-frequency respiratory and cardiac noise. A new method, namely percent amplitude of fluctuation (PerAF), has the best reliability relative to the regional homogeneity, ALFF, and degree centrality (22–24). Therefore, the proposed new method of PerAF may allow us to increase sensitivity and decrease bias when addressing the regional brain activity alternations associated with SD. However, SD has not currently been studied.

Sleep is associated with the gene transcription involved in synthesis and the maintenance of cell membrane lipids and myelin in the brain (25–27) which are particularly susceptible to insufficient sleep (27, 28). Chronic insufficient sleep and chronic stress were found to be associated with several structural changes in the brain (29, 30). Therefore, we hypothesized that SD was associated with widespread functional brain alternations, and these changes could be identified by the proposed PerAF method. To test this hypothesis, the present study utilized PerAF to identify these regional brain alternations in healthy university subjects following 36 h SD relative to a normal sleep status, which may yield insight into the neurobiological mechanisms underlying SD.

MATERIALS AND METHODS

Subjects

A total of 20 healthy university subjects (12 males, 8 females; age, 22.25 ± 1.12 years; education, 12.8 ± 1.01 years) were recruited. All participants met the following criteria as in a previous study (1): good sleeping habits; had not used any stimulants, hypnotic medication, and psychoactive medication for at least the last

TABLE 1 | The PerAF differences between SD status and normal sleep status.

Brain regions of peak coordinates	R/L	BA	Voxel volume (mm^3)	t-score of peak voxels	MNI coordinates
					X, Y, Z
Cerebellum posterior lobe	R	N/A	916	−8.2592	36, −72, −45
Cerebellum posterior lobe	L	N/A	610	−8.1107	−30, −75, −33
Posterior cingulate, lingual gyrus, cuneus	L, R	17, 18	1,347	8.3713	−18, −66, 12
Superior frontal gyrus, medial frontal gyrus	L, R	8, 9	859	−7.6507	−6, 45, 42
Precentral gyrus, postcentral gyrus	L, R	3, 4	1,496	7.6479	15, −36, 72

The statistical threshold was set at a corrected significance level of individual two-tailed voxel-wise $p < 0.001$ using a Gaussian random field corrected threshold of cluster $p < 0.001$ (minimum continuous cluster voxel volumes ≥ 7,101 mm^3).
PerAF, percent amplitude of fluctuation; SD, sleep deprivation; R, right; L, left; BA, Brodmann's area; MNI, Montreal neurological institute; N/A, not applicable.

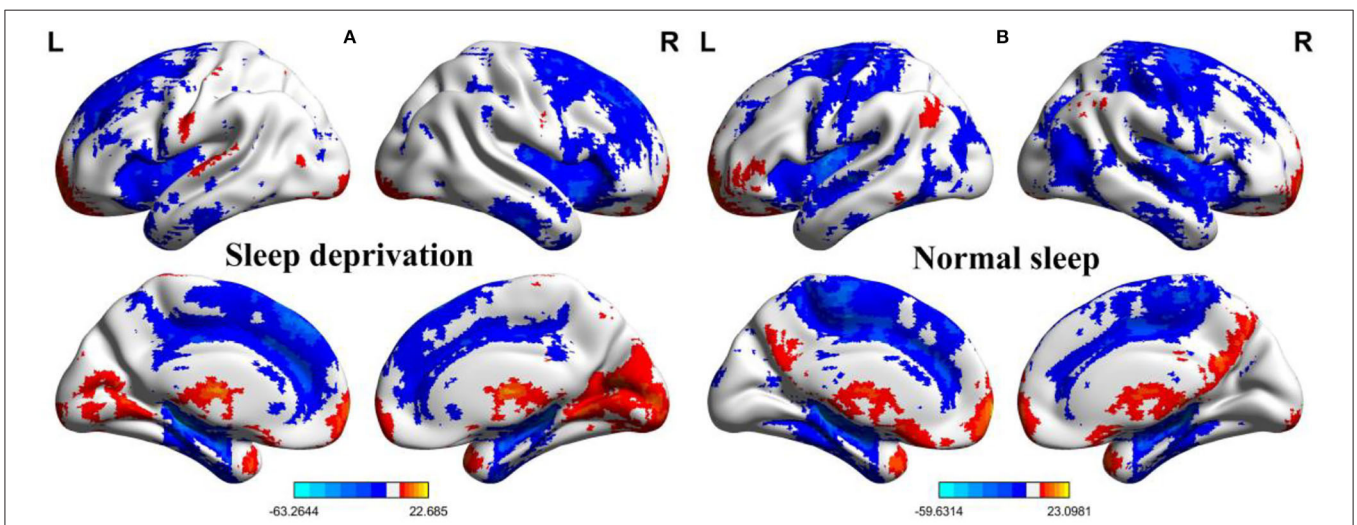

FIGURE 1 | One sample *t*-test differences of SD status and normal sleep status in PerAF maps. **(A)** One sample results for sleep deprivation group; **(B)** One sample results for normal sleep group. SD, sleep deprivation; R, right; L, left; PerAF, percent amplitude of fluctuation.

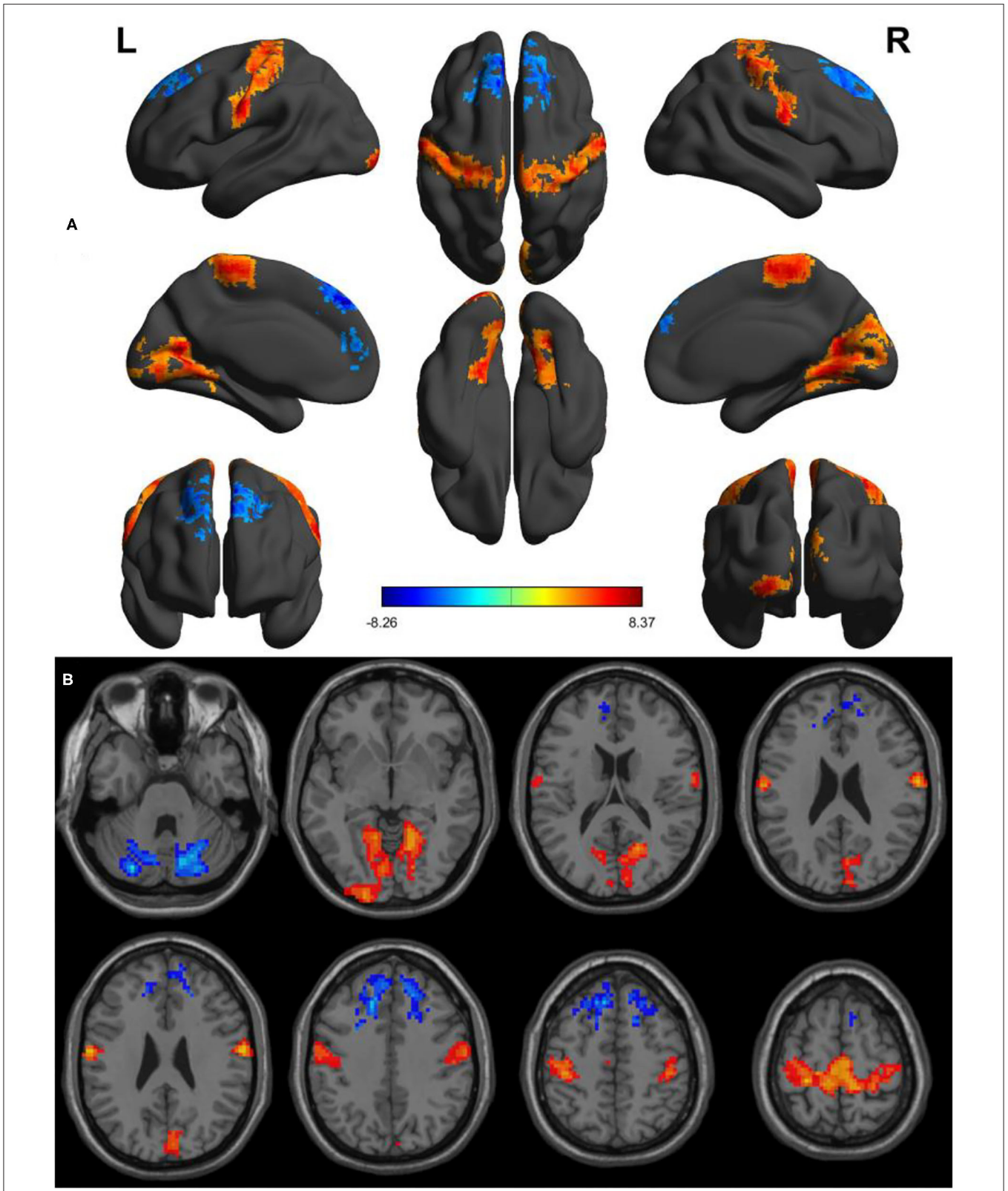

FIGURE 2 | Altered PerAF between SD status and normal sleep status. **(A)** A comprehensive view; **(B)** Axial view. Red color, increased PerAF areas; Blue color, decreased PerAF areas. R, right; L, left; PerAF, percent amplitude of fluctuation; SD, sleep deprivation.

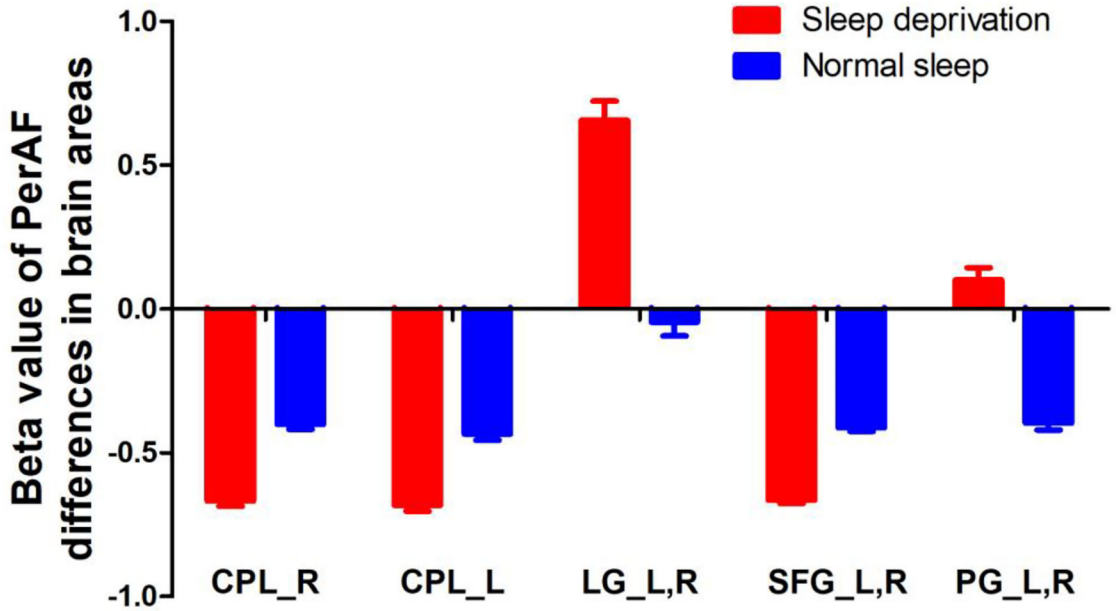

FIGURE 3 | PerAF value of between-group differences in regional brain areas. PerAF, percent amplitude of fluctuation; R, right; L, left; CPL, cerebellum posterior lobe; LG, lingual gyrus; SFG, superior frontal gyrus; PG, postcentral gyrus.

3 months; score of Pittsburgh sleep quality index lower than five, score of Hamilton Depression Rating Scale lower than seven, and score of Hamilton Anxiety Rating Scale lower than seven. The exclusion criteria met the following criteria: any history of pathological brain findings and head trauma; any foreign implants and inborn or acquired diseases; BMI >32 and BMI <19.8; any psychiatric or neurological disorders, substance dependency; and any history of sleep complaints.

The 36 h SD procedure was the same as in previous studies (1, 16), which started at 8:00 p.m. in the evening and ended at 8:00 a.m. on the third day. Our team took turns monitoring the subjects for quality control. All university subjects were required to stay awake and not allowed to sleep during the entire time of the SD procedure. All subjects were not allowed to leave the testing room and were provided with food and water during the SD procedure. All food and/or beverages did not contain caffeine, taurine, or other psychoactive substances that could influence anxiety. This study was approved by the ethical committee of our hospital. All participants were told the purpose, methods, and potential risks, and were asked to complete written informed consent.

Attention Network Test (ANT)

All university subjects were required to conduct an attention network test (ANT) at 8:00 p.m. on the first day and at 8:00 a.m. on the third day before the MRI scans (1, 5, 31, 32). The description of the ANT was the same as in a previous study (1). The ANT comprised of three cue conditions (no cue, center cue, spatial cue) and two arrows (congruent and incongruent) in the center. Participants gave their responses by identifying the congruent or incongruent direction of central arrows. The accuracy rate and reaction time were recorded by calculating the corrected recognition and response times, respectively.

TABLE 2 | ROC curve for PerAF differences in brain areas between SD status and normal sleep status.

Brain area	AUC, 95%CI	Sensitivity, %	Specificity, %	Cut off point[a]
R cerebellum posterior lobe	0.993 (0.976–1)	0.95	0.95	−0.5425
L cerebellum posterior lobe	0.965 (0.918–1)	0.95	0.85	−0.594
Bilateral visual cortex	0.978 (0.942–1)	0.95	0.9	0.247
Bilateral dorsolateral prefrontal cortex	1	1	1	−0.546
Bilateral sensorimotor cortex	0.993 (0.974–1)	1	0.95	−0.2215

[a] *Cut off point of mean PerAF signal value.*

MRI

All subjects underwent MRI scans twice, one following normal sleep and the other following 36 h SD. We used a 3.0-Tesla MR scanner (Prisma, Siemens, Germany) to finish the resting-state fMRI session. Firstly, a total of 176 slices of high-resolution anatomical volumes (repetition time = 1,900 ms, field of view= 256 × 256 mm, echo time = 2.26 ms, thickness = 0.5 mm, gap = 1 mm, flip angle = 11^0, and acquisition matrix = 256 × 256) with sagittal orientation were acquired. Next, a total of 240 functional volumes (repetition time = 2,000 ms, acquisition matrix = 64 ×

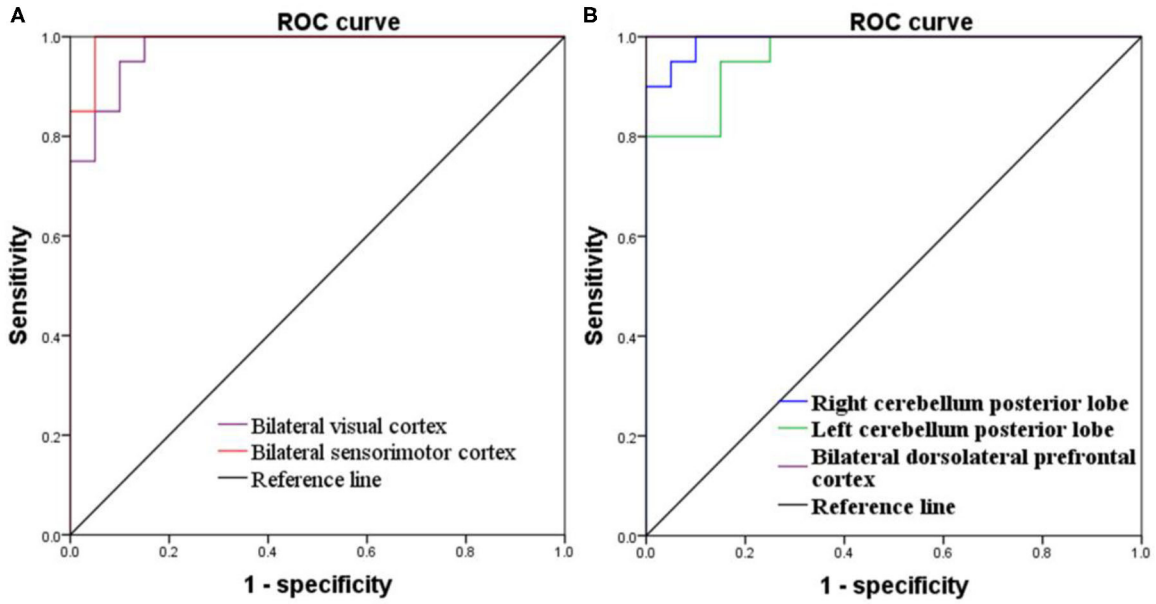

FIGURE 4 | ROC curve of PerAF differences in brain areas. **(A)** Increased PerAF areas; **(B)** Decreased PerAF areas. ROC, receiver operating characteristic; PerAF, percent amplitude of fluctuation.

64, echo time = 30 ms, gap = 0.7 mm, thickness = 3.5 mm, flip angle = 90°, FOV = 224 × 224 mm) were collected.

Data Analysis

Data pre-processing was performed by the RESTplus V1.2 (http://www.restfmri.net) toolbox. Firstly, the first ten functional volumes were removed. Next, other steps including form transformation, slice timing, head motion correction, spatial normalization to Montreal Neurological Institute (MNI) space and re-sampled at a resolution of 3 × 3 × 3 mm^3, smooth (full-width Gaussian kernel = 6 × 6 × 6 mm) and linear detrend and filter (0.01–0.08 Hz) (21). Participants with more than 1.5 mm maximum translation and/or more than 1.5° degree of motion rotations in any directions were removed. Friston's 24 head motion parameters were used as covariates to regress out the effects of head motion (33–36). Linear regression was used to remove the covariates of global mean signal, white matter, head-motion, and cerebrospinal fluid signal. The PerAF method is the percentage of resting-state frequency domain of the blood oxygen level-dependent signal relative to the mean signal intensity of one given time series. After these steps of data pre-processing, the PerAF method was calculated, thus generating PerAF, mPerAF, and the z-transformation of zPerAF.

Statistical Analysis

Two pair *t*-tests were used to calculate the ANT differences between SD status and normal sleep status by IBM SPSS 21.0. A threshold of $p < 0.05$ was considered as significant.

Firstly, one sample *t*-tests were applied to calculate the within-group differences in brain areas for sleep deprivation status and normal sleep status, separately [false discovery rate (FDR) correction, voxel-wise $p < 0.001$, and cluster-level $p < 0.001$]. Next, a two-pair *t*-test was applied to calculate between-group differences of PerAF in brain alterations [gaussian random field (GRF) correction, voxel-wise $p < 0.001$ and cluster-level $p < 0.001$, cluster voxel volumes ≥ 7,101 mm^3]. A receiver operating characteristic (ROC) curve was frequently applied to test if neuroimaging methods might serve as potential neurobiological indicators to differentiate the two different groups (5, 16, 19, 20, 37). Here, the ROC was applied to identify the ability of the proposed PerAF method in distinguishing SD status from normal sleep status. The relationships between SD-induced brain alterations and attention deficits were determined by Pearson correlation analysis. A threshold of $p < 0.05$ was considered as significant.

RESULTS

Sample Characteristics

Compared with normal sleep, acute SD showed a 2.23% poorer accuracy rate (normal sleep status, accuracy rate = 98.32 ± 1.54%; SD status, accuracy rate = 96.13 ± 2.93%; $t = -2.961$; $p = 0.005$), and an 8.82% slower reaction time (normal sleep status, reaction time = 540.5 ± 58.09 ms; SD status, reaction time = 588.16 ± 70.84 ms; $t = 2.327$; $p = 0.025$).

PerAF Differences

Within-group differences for the SD status (**Figure 1A**) and the normal sleep status (**Figure 1B**) are shown in **Figure 1** ($p < 0.001$, FDR corrected). The within-group statistical maps showed that the covered locations of the PerAF differences

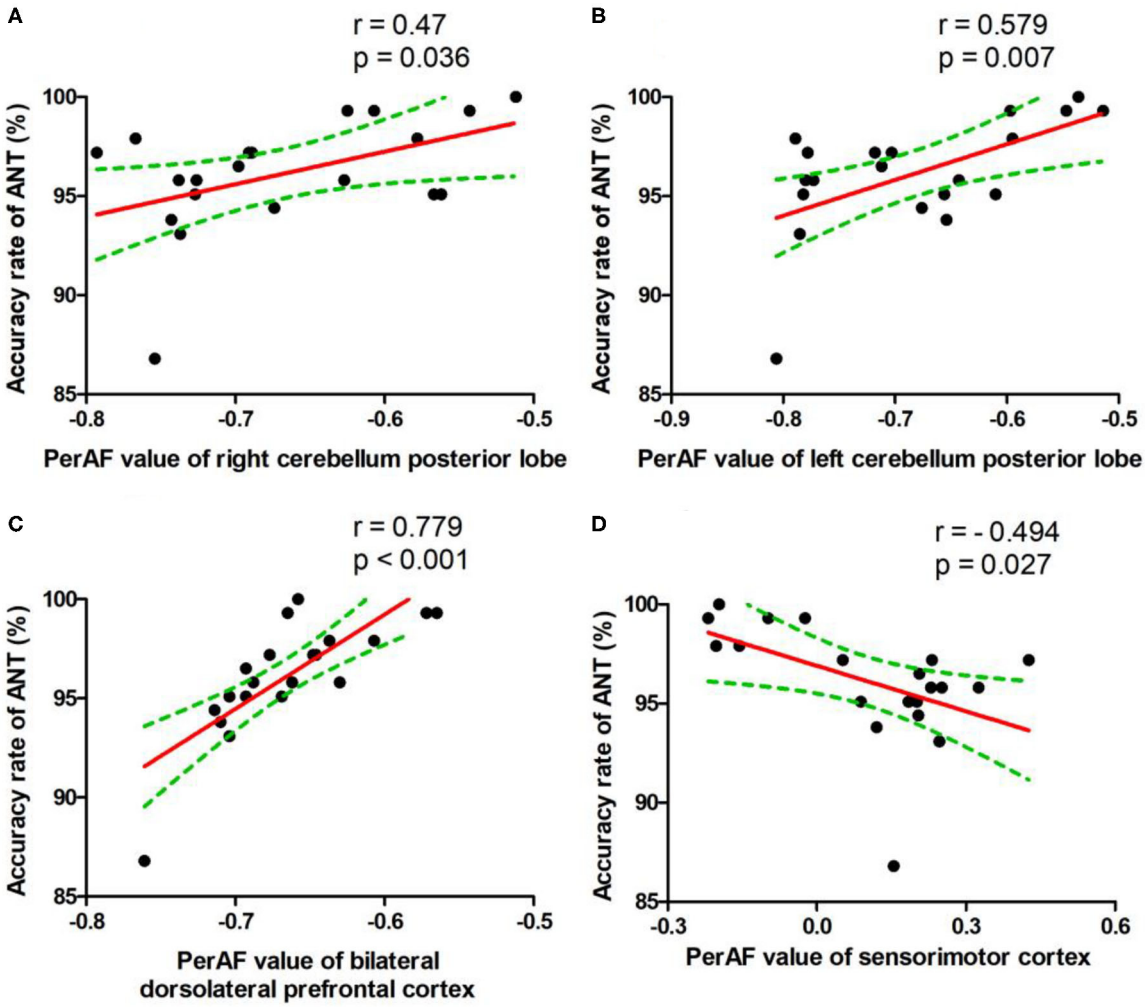

FIGURE 5 | Pearson's correlation. Correlations were found between accuracy rate of ANT and bilateral cerebellum posterior lobe (Right, **A**; Left, **B**), bilateral dorsolateral prefrontal cortex **(C)**, or bilateral sensorimotor cortex **(D)**. PerAF, percent amplitude of fluctuation; ANT, attention network test.

in brain areas during SD status (**Figure 1A**) were smaller than that of during normal sleep status (**Figure 1B**). The between-group statistical maps showed that compared with normal sleep status, SD status showed increased PerAF differences in the bilateral visual cortex (BA 17, 18) and bilateral sensorimotor cortex (BA 3, 4), and decreased PerAF differences in the bilateral dorsolateral prefrontal cortex (BA 9) and bilateral cerebellum posterior lobe (**Table 1**, **Figures 2A,B**).

ROC Curve

Since these SD-induced brain alterations exhibited differences between the SD status and normal sleep status they might serve as potential neurobiological indicators to differentiate the two different sleep statuses, we extracted the mean PerAF values of these areas for ROC curve analysis (**Figure 3**). Our data indicated that these areas revealed an extremely high discriminatory power with a high AUC value of 0.986 ± 0.01 (0.993–1), and further diagnostic analysis also showed a high degree of sensitivity (97 ± 2.74%, 95–100%) and specificity (93 ± 5.7%, 85–100%) (**Table 2**, **Figures 4A,B**).

Pearson Correlation Analysis

During the SD status, several correlation analyses between the ANT differences and the SD-induced brain alterations that exhibited differences between the SD status and normal sleep status were reported (**Figures 5A–D**). The accuracy rate of the ANT positively correlated with the bilateral cerebellum posterior lobe (Right, $r = 0.47$, $p = 0.036$, **Figure 5A**; Left, $r = 0.579$, $p = 0.007$, **Figure 5B**) and the bilateral dorsolateral prefrontal cortex ($r = 0.779$, $p < 0.001$, **Figure 5C**), respectively. Furthermore, the accuracy rate of the ANT negatively correlated with the bilateral sensorimotor cortex ($r = -0.494$, $p = 0.027$, **Figure 5D**).

DISCUSSION

The present study is the first to apply the proposed PerAF method to identify SD-induced brain alterations in healthy university

subjects following a total of 36 h of SD, and their relationships with the ANT. The present study reported three main findings: (a) the status transformation from normal sleep to 36 h SD in healthy subjects resulted in a 2.23% decrease in accuracy rate and an 8.82% increase in reaction time in attention; (b) SD was associated with increased PerAF differences in the bilateral visual cortex and bilateral sensorimotor cortex, and decreased PerAF differences in the bilateral dorsolateral prefrontal cortex and bilateral cerebellum posterior lobe; (c) these SD-induced brain areas exhibited an extremely high discriminatory power with extremely high AUC values (0.993–1) in distinguishing the two sleep statuses, which indicated that the PerAF method might be a potential neuroimaging indicator to differentiate different sleep statuses; (d) the accuracy rate positively correlated with the bilateral cerebellum posterior lobe and bilateral dorsolateral prefrontal cortex, and negatively correlated with the bilateral sensorimotor cortex.

Previous neuroimaging studies have shown higher regional spontaneous neural activity and short-range functional connectivity in the visual cortex in chronic insomnia patients and healthy individuals after sleep deprivation (4, 16, 18, 19, 38–41). The hyper-responses of the visual cortex has been considered as a core factor leading to the inability to initiate or maintain sleep in chronic insomnia patients (19, 39, 40, 42–46). Increased regional brain activity and functional connectivity, such as voxel-mirrored homotopic connectivity and short/long-range functional connectivity, and decreased gray matter volumes in the somatosensory cortex in chronic insomnia patients and in healthy individuals after SD status have been reported (1, 16, 37, 41). These regional brain alterations had several correlations with attention and spatial working memory deficits after the SD session (1). PET studies found that SD increased the metabolic rate of glucose in the visual and somatosensory cortex, and the metabolic rate was higher after a longer duration of SD than that of a shorter duration of SD (47, 48). Our data supported these findings. In our study, SD increased PerAF differences in the two cortexes, and these areas negatively correlated with the accuracy rate of the ANT. In this framework, we speculated that increased PerAF in these regions might be compensatory responses to the cognitive deficits. These findings support the excessive hyperarousal theory of insomnia (45).

The cerebellum posterior lobe is associated with language, cognition, and emotion, and also with the regulation of planning, initiating, and coordinating movement (4, 19, 49–51). Previous studies have shown that the cerebellum was associated with several neurologic and psychiatric diseases, including obstructive sleep apnea (52), depression (53), primary insomnia (19, 39), mood disorders (54), and sleep deprivation (4, 16, 41) and was correlated with the accuracy rate of ANT (16). Our study showed consistent findings with decreased PerAF in the cerebellum and dorsolateral prefrontal cortex, and the two areas showed positive correlations with the accuracy rate of ANT. In this framework, the decreased regional brain activity in the cerebellum and dorsolateral prefrontal cortex may suggest that the brain needs to recruit more advanced cognitive function-related brain areas to offset the attention deficits during SD, due to a continuing declined activity in these two areas during insufficient sleep status (16).

CONCLUSIONS

In summary, the proposed method of PerAF might be a potential sensitivity neuroimaging indicator to differentiate different sleep statuses. Acute SD could lead to an ∼8% attention deficit, which was associated with the increased PerAF differences in the visual cortex and sensorimotor cortex, and the decreased PerAF differences in the dorsolateral attention cortex and cerebellum. These findings could expand our knowledge of the pathophysiological mechanism of insufficient sleep and related diseases, and could provide guidance for healthcare professionals to reduce the mistakes caused by lack of sleep. However, there are several limitations that should be addressed. Firstly, the effect of differences in gender was not considered (4, 19). Secondly, the small sample sizes limited the comparisons. Thirdly, the caloric intake and sleep at baseline were not considered.

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 Jiangxi Provincial People's Hospital Affiliated to Nanchang University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

BZ, ZiL, and HZ conceived and designed the whole experiment. BZ, JZ, ZoL, and PY took responsibility for the integrity of the data, the accuracy of the data analysis, and statistical data analysis. BZ wrote the main manuscript text, and under took the critical interpretation of the data. All authors contributed to the final version of the paper and have read, as well as, approved the final manuscript.

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Does Homeostatic Sleep Pressure Buildup Explain Objective Excessive Daytime Sleepiness in Adults With ADHD? An Exploratory Study

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Background: Excessive daytime sleepiness (EDS) is central in Attention deficit hyperactivity disorder (ADHD) but its causes remain unclear. The aim of this study was to explore objective EDS and homeostatic sleep pressure buildup, evaluated by power theta–alpha frequency (PTAF), in drug-free sleepy adults with ADHD and controls.

Methods: Participants were placed during a 36-h period of extended wakefulness under constant routine protocol to strictly control sleep time, sleep duration, and circadian zeitgebers.

Results: Eight drug-free sleepy patients with ADHD and 7 matched controls were included. The ADHD group had significantly shorter sleep latency on the Maintenance of Wakefulness Test (MWT) throughout extended wakefulness than the control group. There was no significant difference between the groups in PTAF evolution during extended wakefulness and in kinetic sleep pressure buildup, evaluated by the time constant of saturating exponential function.

Limitations: The sample was small, so the findings cannot be generalized. Moreover, psychiatric comorbidities and circadian regulation should be taken into account in future studies.

Conclusion: In very controlled conditions, mean sleep latency on the MWT during the whole extended wakefulness was significantly shorter in sleepy patients with ADHD than in control subjects. However, the difficulty to remain awake during soporific circumstances observed in these patients with ADHD cannot be explained by changes in the kinetic of sleep pressure buildup.

Clinical Trials Registration: www.clinicaltrials.gov/, Identifier: NCT02217371.

Keywords: ADHD (attention deficit and hyperactivity disorder), sleepiness, sleep pressure, maintenance of wakefulness test, phenotype

INTRODUCTION

Attention deficit hyperactivity disorder (ADHD) is the most common developmental disorder and is characterized by inappropriate levels of inattention, impulsivity, and hyperactivity (1). Follow-up studies have documented the persistence of ADHD into adulthood in 50–65 % of cases (2). In the USA, the prevalence of ADHD in adults has been estimated at 4.4 % (3). ADHD significantly affects major life domains, notably social and occupational functioning. Excessive daytime sleepiness (EDS) is an interesting focus within the context of ADHD in adults as shown in studies using an automatic resting EEG classification of sleepiness (VIGALL, 7 EEG-vigilance stages) who demonstrated unstable arousal regulation in children and adults with ADHD. This arousal instability was characterized by a faster decline to the low EEG-vigilance stages and more fluctuations in their stages of vigilance (4, 5). Moreover, in a study (6) conducted on a population of regularly registered highways drivers: those with ADHD symptoms expressed greater EDS than those who did not have these. This result was confirmed by Ito et al. (7), who showed in a web-based study that the prevalence and severity of EDS in Japanese adults with possible ADHD were higher than in individuals classified as non-ADHD. ADHD is highly associated with primary sleep disorders (8), which could explain the EDS. However, Bioulac et al. (9) found that a significant proportion of adults with ADHD free of primary sleep disorders exhibit objective EDS. Therefore, the cause of objective EDS in ADHD in adults remains elusive and is probably not unique.

Consistent evidence for circadian rhythm disruption in ADHD is now emerging. As described by Coogan and McGowan in their recent systematic review (10), ADHD is associated with evening chronotype and with delayed sleep onset with an objectively measured prevalence of 73–78% in both children and adults with ADHD (11, 12). Delayed sleep onset, or delayed sleep phase syndrome (DSPS), induces a misalignment between circadian time and social time known as social jetlag (13). The social jetlag observed mostly in evening chronotype is associated with sleep restriction, resulting in accumulated EDS over time (13, 14).

Moreover, DSPS may involve changes in the circadian clock drive (Process-C) and/or the sleep homeostatic process (Process-S) (15). Circadian disturbances include an increase in the circadian period, a shorter phase angle, and the dampening of rhythms clock gene expression.

Concerning process-S, we previously demonstrated that evening chronotype feels sleepier during the daytime and that the kinetics of homeostatic sleep pressure build-up differ between morning and evening chronotypes (16). Our hypothesis is that changes in homeostatic sleep pressure build-up could explain EDS in the sleepy adults ADHD subgroup.

To go further than the aforementioned studies, the aim of this study was to explore EDS objectified by MWT and homeostatic sleep pressure in a sleepy ADHD subgroup, and in control subjects for a 36-h period of extended wakefulness under constant routine protocol.

METHODS AND MATERIALS

Population

Patients with ADHD were recruited from the sleep clinic at the University Hospital in Bordeaux (France) according to the *DSM-IVR* criteria (17). The clinical diagnosis of ADHD was validated by standardized instruments. Childhood ADHD and the chronic course of ADHD symptoms from childhood to adulthood were established by a board-certified psychiatrist who carried out a clinical evaluation and administered a semi-structured diagnostic interview (Conners' Adult ADHD Diagnostic Interview for *DSM-IV*, CAADID) (18). The Conners' Adult ADHD Rating Scales (CAARS), observer form, was used. Patients with ADHD were included if they presented a mean sleep latency < 20 min on the Maintenance of Wakefulness Test (MWT) (four MWT trials at 2-h intervals, 10 am, 12 am, 2 pm, 4 pm) (9). According to the American Academy of Sleep Medicine (AASM), the MWT may be used to assess an individual's ability to remain awake when his or her inability to remain awake constitutes a public or personal safety issue (19).

We excluded all patients with any clinically relevant medical or psychiatric condition including current affective or psychotic disorders, substance abuse within 1 year prior to screening, and long-term treatment with benzodiazepine. Comorbid psychiatric disorders were assessed with the Mini International Neuropsychiatric Interview (MINI 5.0.0., *DSM IV*).

All patients were withdrawn from psychostimulant medication for a minimum of 72 h before starting the study.

Healthy control subjects were recruited from the general population and were matched with ADHD patients according to age ($\pm$5 years), sex and chronotype, as defined by the score on the Morning Evening Questionnaire (MEQ) (20). We excluded subjects with any psychiatric disorders or any complaint of sleep disorder [reported on the Basic Nordic Sleep Questionnaire (BNSQ)] (21), or an Epworth Sleepiness scale score > 10 (22). ADHD symptoms were ruled out in the control population with the Wender Utah Rating Scale for ADHD (23), ASRS (Adult ADHD Self-Report Scale) (18 items) (24). Controls were included if they presented a mean sleep latency > 36 min on the MWT (four MWT trials at 2-h intervals).

For patients and controls, the presence of nocturnal sleep-disordered breathing (AHI > 10/h) and periodic limb movements (index > 15/h) were ruled out with ambulatory polygraphy. Patients and controls with restless leg syndrome and DSPS were also excluded. Subjects provided written informed consent, and the local ethics committee [consultative committee for the protection of persons participating in biomedical research (CPP Sud-Ouest et Outre-Mer III)] approved the study. The study is registered at Clinicaltrials.gov (NCT02217371).

Study Design

For 4 days prior to the study, participants were asked to maintain regular bedtimes and wake-up times according to their usual preferences. During this period, compliance was checked by sleep diaries and actimetry. They performed a nocturnal polysomnography (PSG) just before the constant routine to control sleep time and duration. The next morning, 1 h after

their preferential wake-up time, all volunteers underwent a 36-h period of extended wakefulness in the constant routine protocol (25). As sleep pressure is generated by the interplay of circadian, homeostatic drives and preferential social timing (16, 26), the night before the constant routine, subjects slept according to their individual and preferential sleep schedule (bedtime and wake time) in order to not modify the kinetic of sleep pressure. Volunteers were kept in a constant semi-recumbent posture in bed and were restricted to very low activity levels, under dim light (<50 lux) and received hourly snacks throughout the day and night.

Sleep pressure was evaluated by theta-alpha (6–9 Hz) band of frontal EEG (27) during the Karolinska Drowsiness Test (4-min eyes-open session, KDT). Frontal power theta–alpha frequency (fPTAF) was calculated (Hanning window) by 4-s epochs after an automatic artifact rejection (ACUTE software, Physip France). Kinetics of sleep pressure buildup were defined by asymptote and time constant assessed by saturating exponential function (27). The first MWT trial began about 1H20 after the subject preferential wake-up time. MWT trials were performed every 4 h (after 1H20 (T1), 5H20 (T2), 9H20 (T3), 13H20 (T4), 17H20 (T5), 21H20 (T6), 25H20 (T7), 29H20 (T8), and 33H20 (T9) of wakefulness). Subjects followed classical MWT instructions (28). Trials were ended after 40 min if no sleep occurred or after three consecutive epochs of stage 1 sleep or after one epoch of any other stage of sleep. Sleep latency was defined as the first epoch of any stage of sleep.

During PSG, KDT and MWT, EEG, EOG, and EMG were recorded on a Brainbox EEG-1042 Amplifier with coherence software (Natus, France). All electrophysiological signals were digitized at a sampling rate of 256 Hz. KDT and MWT were repeated every 4 h.

STATISTICAL ANALYSIS

Actimetry and PSG measures were compared between ADHD patients and controls using Wilcoxon rank tests. Repeated outcomes (MWT and Process S fPTAF) as well as the constant and the asymptote of the fPTAF were compared between groups using linear mixed effects models adjusted for age, gender, and Hörne and Ostberg score, and taking into account an interaction between group and time. The analyses were conducted on available data, using SAS software v9.4 (SAS Institute Inc., Cary, NC, USA). *p*-Values <0.05 were considered statistically significant.

RESULTS

Population

Eight ADHD patients and 9 control participants were recruited. Two control participants withdrew from the experiment during the constant routine protocol. The sample thus consisted of 15 subjects: 8 ADHD patients (mean age = 39.8 ± 11 years, range 21–53 years, BMI = 24.6 ± 4.7, 2 males) and 7 control participants (mean age = 42.6 ± 9.1, range 25–53, BMI = 24.0 ± 3.8, 1 male). The mean MEQ score in ADHD patients was 56.8 ± 12.7, demonstrating that sleepy ADHD patients were mostly intermediate chronotypes. Only one sleepy ADHD patient was classified as evening chronotype.

TABLE 1 | Demographic and clinical characteristics (Mean ± SD) of sleepy ADHD patients and control participants at inclusion.

	ADHD patients ($n = 8$)	Controls ($n = 7$)
Demographic and clinical characteristics		
Age (years)	39.8 ± 11.0	42.6 ± 9.1
Gender (% females)	75%	85.7%
BMI (kg/m^2)	24.6 ± 4.7	24.0 ± 3.8
Mean sleep latency on MWT (min)	13.9 ± 4.5	38.9 ± 2.0
Self-reported questionnaires		
MEQ score	56.8 ± 12.7	57.9 ± 2.8
ESS score	16.9 ± 2.4	3.4 ± 2.5
WURS score	55.5 ± 9.4	10.6 ± 7.6

ADHD, attention deficit/hyperactivity disorder; BMI, body mass index; MWT, maintenance of wakefulness test; MEQ, morningness–eveningness questionnaire; ESS, Epworth Sleepiness scale; WURS, Wender Utah Rating Scale.

Among the 8 patients with ADHD included, 50% presented a psychiatric comorbidity (25% had comorbid anxiety disorder (past or present) and 50% had a history of mood disorders but no current mood disorder). Fifty percent presented with ADHD of the mixed subtype and 50% with the inattentive subtype. Patients with ADHD presented the following scores on the CAARS (58.4 ± 15.7) and the Brown questionnaire (69.8 ± 26.3).

Table 1 shows the demographic and clinical characteristics of ADHD patients and controls.

Actimetry

There was no significant difference between patients with ADHD and controls in total sleep time for the four nights before the protocol (Night 1: 422.6 ± 176.0 min vs. 428.0 ± 36.2 min, p =0.41; Night 2: 453.3 ± 128.2 min vs. 437.0 ± 52.2 min, $p = 0.73$; Night 3: 419.8 ± 63.9 min vs. 385.3 ± 73.6 min, $p = 0.39$; Night 4: 429.3 ±110.5 vs. 415.7± 40.2, $p = 0.73$).

Polysomnography

There was no significant difference between patients with ADHD and controls in total sleep time on PSG the night before the protocol: 404.8 (50.0) min vs. 392.9 (62.0) $p = 0.69$.

MWT

The ADHD group had a shorter sleep latency on the MWT than the control group at T1 [34.4 ± 8.7 min vs. 40.0 ± 0.0 min (p = 0.013)]. This significantly shorter sleep latency on the MWT persists over time, but remains stable over the eight measures (p = 0.94) (**Figure 1**).

Process S

There was no significant difference in fPTAF between the groups, whether at T1 (126.5 ± 173.2 vs. 95.9 ± 101.4 ($p = 0.80$), for ADHD patients and controls, respectively) or regarding the evolution of the eight other measures ($p = 0.37$) (**Figure 2**).

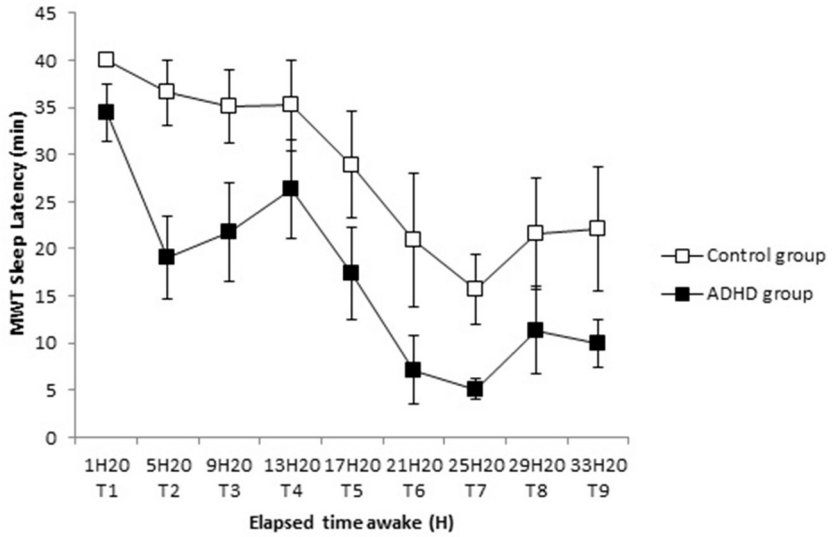

FIGURE 1 | Time course of sleep latency on MWT (Mean ± SE) in sleepy ADHD group and control group during 36-h of sustained wakefulness from T1 to T9.

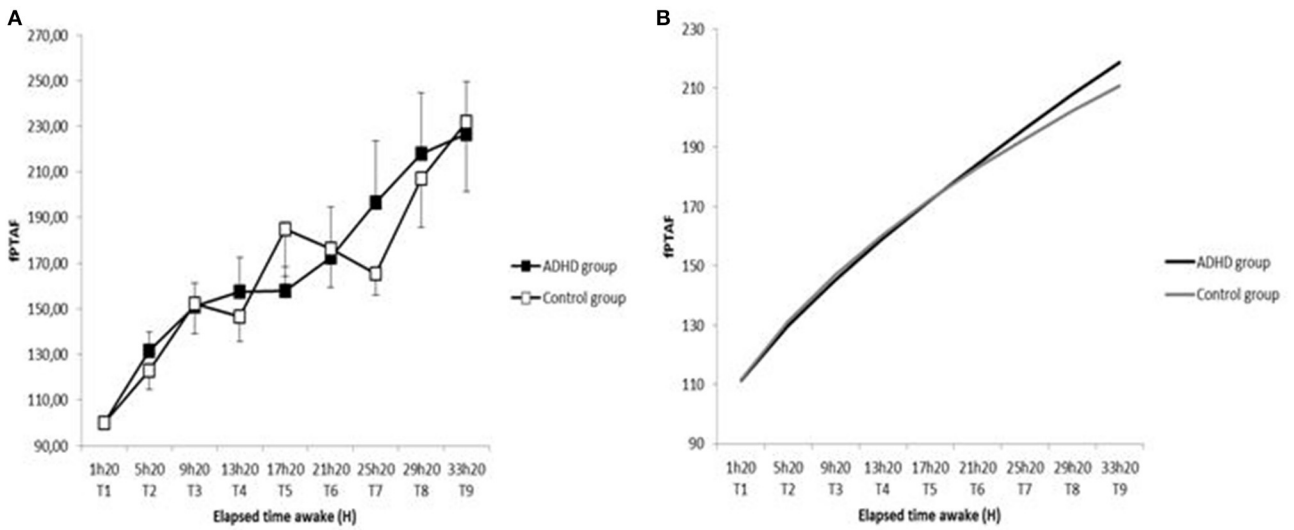

FIGURE 2 | Time course of fPTAF in sleepy ADHD group and control group during 36-h of sustained wakefulness from T1 to T9 **(A)** and sleep pressure buildup in sleepy ADHD and controls assessed by saturating exponential function **(B)**.

Regarding kinetics of sleep pressure buildup, there was no significant difference between the groups in mean time constant (33,043.4 ± 68,519.6 h for ADHD patients and 126,303.9 ± 187,901.4 h for controls; $p = 0.12$), nor in mean asymptote (112,874.9 ± 206,194.4 for TDAH and 534,873.4 ± 10,004,871.6 for controls; $p = 0.18$).

DISCUSSION

This study is the first to explore the impact of sustained wakefulness on objective EDS in drug-free sleepy adults with ADHD during a 36-h period under constant routine. In highly controlled conditions (sleep time, sleep duration and circadian zeitgebers), mean sleep latency on MWT remained significantly shorter in sleepy patients with ADHD than in control participants. Indeed, at the beginning of the protocol (first MWT), sleepy patients with ADHD displayed a significantly shorter mean sleep latency on the MWT than control participants and this difference persists throughout the whole constant routine remaining stable. Contrary to our original hypothesis, difficulty to remain awake during soporific circumstances cannot also be explained by a change in homeostatic sleep pressure since the kinetics of sleep pressure build-up did not differ between ADHD patients and control subjects.

This kind of protocol permits to test fundamental hypothesis about the origin of sleepiness in this subgroup of patients with ADHD. EDS is sometimes caused by insufficient sleep, a delay in the sleep phase (29) or by misalignment between circadian time and social time (social jetlag) (13). As all participants were matched in chronotype and were asked to follow regular and preferred sleep-wake schedules and habitual sleep duration (compliance verified by actimetry) during the 4 days before the constant routine, the EDS observed in our sleepy patients with ADHD cannot have been artificially induced by sleep restriction or social jetlag. Importantly, total sleep time did not differ between the two groups before the constant routine on actimetry and PSG.

These findings suggest that EDS in ADHD may have a central origin, leading to a state of hypo-arousal state, as in narcolepsy. Miano et al. identified five specific sleep phenotypes in ADHD children (30) including one called "narcolepsy-like phenotype" that could correspond to our sleepy ADHD subjects. This hypothesis is supported by the recent work of Diaz-Roman et al. (31), who found shorter REM latency and higher levels of EDS in children with ADHD than in control children. These data might indicate early signs or shared symptoms of narcolepsy in these children with ADHD. In support of a continuum between ADHD and central hypersomnia, Lopez et al. (32) found a high frequency of ADHD and ADHD-like symptoms in patients with central hypersomnia, explaining high levels of EDS and hypersomnolence in adults with ADHD. In a new model of sleep/wake regulation, Fulcher et al. (33) extended the model of Phillips and Robinson (34) by demonstrating that the dynamics of sleep and wake may be controlled not only by circadian rhythms and homeostatic drive but also by orexin levels. The model posits that a reduction in orexin levels leads to reduced daytime arousal without altering any other drives, as observed in our sleepy ADHD subgroup.

Moreover, we cannot exclude the effect of circadian changes associated with ADHD especially the phase delay of circadian phase (11, 35) on EDS even if the patients with ADHD included in this study were classified mostly in intermediate chronotype. Although it is still not certain whether circadian amplitude is impaired in ADHD patients, further studies will have to fully explore changes in circadian phase, circadian period, circadian entrainment, and/or polymorphisms in genes controlling the circadian drive.

This study has several limitations. First, the sample was small given the heaviness of the protocol, so the findings cannot be generalized. Consequently, we could not take *DSM-IV* subtypes into account. Second, our patients had psychiatric comorbidities, half of them suffering from another psychiatric disorder, as expected. A limit of our study is that we did not control for stressful life events which typically can be associated with reduced sleep causing daytime sleepiness. Therefore, the EDS in our patients with ADHD may have been influenced by psychiatric comorbidities. Moreover, we cannot exclude the effect of circadian changes associated with ADHD especially the phase delay of circadian phase on EDS even if the patients with ADHD included in this study were classified mostly in intermediate chronotype. Although it is still not certain whether circadian amplitude is impaired in ADHD patients (10), further studies will have to fully explore changes in circadian phase, circadian period, circadian entrainment, and/or polymorphisms in genes controlling the circadian drive. Finally, we cannot totally exclude an after effect over 72 h due to withdrawal-related hypersomnia symptoms (36). However, in the literature, most of ADHD-related studies commonly stopped psychostimulants treatment for a period of 48–72 h before the protocol (37, 38). Further studies will have to control this limiting factor.

In conclusion, this study conducted in highly controlled conditions revealed a significant and stable difference in mean sleep latency on the MWT between drug-free sleepy patients with ADHD and control subjects. The difference cannot be explained by specific changes in the kinetics of sleep pressure buildup with ADHD. EDS is a key objective biomarker to better evaluate ADHD patients. The clinical implications are that personalized pharmacologic treatment with wakefulness-promoting drugs could improve cognition and behaviors in sleepy ADHD patients.

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 Consultative Committee for the protection of persons participating in biomedical research [CPP Sud-Ouest et Outre-Mer III—Clinical Trials Registration: NCT02217371]. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

SB, PS, PP, and JT: designed this study. SB, PS, JT, ET, AB, CB, and MB: coordinated the data collection and enrolment of participants. SB, PS, and JT drafted the manuscript which was added to and modified by AB, ET, CB, MB, and PP. All authors read and approved the final manuscript.

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Relation of Decreased Functional Connectivity Between Left Thalamus and Left Inferior Frontal Gyrus to Emotion Changes Following Acute Sleep Deprivation

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Objective: The thalamus is a key node for sleep-wake pathway gate switching during acute sleep deprivation (ASD), and studies have shown that it plays a certain role in emotion changes. However, there are no studies on the association between the thalamus and emotion changes in ASD. In this study, we used resting-state functional magnetic resonance imaging (R-fMRI) to explore whether changes in the functional connections between the thalamus and other brain regions are related to emotion changes and further explored the function of the thalamus under total ASD conditions.

Method: Thirty healthy, right-handed adult men underwent emotional assessment according to the Profile of Mood States Scale and R-fMRI scans before and after ASD. The correlations between changes in functional connectivity between the thalamus and other brain regions and emotion changes were then studied.

Results: Positive emotions and psychomotor performance were reduced, and negative emotions were increased following ASD. The functional connections between the left thalamus and left middle temporal gyrus, left inferior frontal gyrus, right thalamus, right inferior temporal gyrus, left middle temporal pole gyrus, right calcarine, left cuneus, left rectus and left medial superior frontal gyrus were significantly altered. Decreased functional connectivity between left thalamus and left inferior frontal gyrus related to emotion changes following ASD.

Conclusion: This study finds that functional changes in the thalamus are associated with emotion changes during ASD, suggesting that the left thalamus probably plays an essential role in emotion changes under ASD conditions.

Keywords: mood, functional connectivity, resting-state functional magnetic resonance imaging, thalamus, inferior frontal gyrus, acute sleep deprivation

INTRODUCTION

"Sleep deprivation" can be summarized as less sleep than is usually required. It can be acute or chronic, with the effects of a small amount of sleep deprivation accumulating over days, weeks or longer (1). With the rapid development of social modernization, people often actively or passively suffer from the adverse effects of chronic sleep deprivation (CSD) and acute sleep deprivation (ASD) (2, 3). Sleep deprivation leads to a series of neurological and behavioral changes that can significantly interfere with the brain's cognitive and emotional abilities. The short-term consequences of sleep deprivation include increased stress response, physical pain, decreased quality of life, emotional disorders and performance impairment (4), while the long-term consequences of sleep deprivation in healthy individuals include cardiovascular disease, weight-related problems, metabolic syndrome, dyslipidemia, and colorectal cancer (4). There is also an increase in all-cause mortality among men suffering from sleep deprivation (4). Furthermore, following sleep deprivation, brain function is significantly impaired in terms of attention, decision-making, spatial navigation, working memory and emotional and social processing (5, 6).

It is well known that sleep plays a critical role in emotional processing and regulation (5). Functional magnetic resonance imaging (fMRI) studies have shown that sleep-deprived people have altered emotional brain networks, mainly in the limbic system (5). Compared with healthy people, the volume, activity and functional connections of the amygdala, insula, cingulate area and prefrontal lobe in patients with emotional disorders, such as various types of anxiety and bipolar disorder, are significantly changed, which further confirms that these are the main brain areas responsible for the related emotions (7–9). Correlations between these brain regions and emotion have mainly been identified in studies of people with CSD, but a growing number of studies show that ASD has a wide range of effects on emotion (10, 11). Further research shows that the amygdala, anterior insula, medial prefrontal cortex and anterior cingulate cortex are also significantly altered under ASD, and associated with emotion changes caused by ASD (6, 12). The above research findings provide preliminary evidence that the areas of the brain associated with emotion changes are largely the same in both ASD and CSD.

All brain regions are affected by emotions (13). However, the research on human brain by fMRI mainly focuses on the higher cortical regions, and there are few studies on the involvement of subcortical structures in emotional changes. As an important structure involved in the sleep-wake pathway, the thalamus has been shown to be involved in alert-related brain cognitive functions (6). And in certain chronic progressive diseases such as anxiety disorder and insomnia, the thalamus is involved in the emotional neural networks. Research into the emotional dysregulation circuit shows that the thalamus plays a particular role in emotion changes (14, 15). Furthermore, studies have shown that CSD (insomnia, etc.) can cause functional changes in the thalamic-emotional core region (16, 17). To date, the emotional role of the chronically sleep-deprived thalamus has been documented, but the fact that the thalamus is involved in emotion changes has not been adequately studied. At the same time, there were almost no studies have investigated the emotional role of the thalamus during ASD. While the vast majority of studies show that the thalamus plays a key central role in the sleep-wake pathway and involved in a variety of brain cognitive functions (6, 18–21), and the thalamus is located anatomically in the core area of the brain, Therefore, there is good evidence to speculate that the thalamus may play a unique role in emotion changes caused by ASD. However, ASD and insomnia show extensive changes in the microstructure of gray matter and share common but different neurobiological characteristics in brain morphology (22). It is necessary to provide direct evidence to test this hypothesis.

We hypothesized that the functional connections between the thalamus and brain regions proven to be associated with emotion would be altered following ASD, and the functional changes between the thalamus and other regions would correlate with the emotion changes. To test this hypothesis, we devised a within-subject statistical design of 36 h total ASD, then used f-MRI to assess whether changes in the functional connections between the thalamus and other brain regions were correlated with emotion changes under ASD conditions.

MATERIALS AND METHODS

Participants

We recruited 30 young male college students (right-handed, age range: 20–30 years) and offered them a financial reward to participate in this study. During the recruitment process, we explained in detail the main purpose of the study and its whole process, as well as the possible risks and countermeasures. All subjects voluntarily signed their informed consent. Prior to the beginning of the experiment, we invited qualified Chinese specialists to conduct standardized physical examinations. The main forms of medical examination were subjective and objective physical examinations, as well as related self-reporting scale tests, to exclude subjects who may have serious diseases or be at risk of accidents during the experiment. The inclusion criteria we set were as follows: ① No disease history of the circulatory system or respiratory system, no nervous system structure or function impairment, and no history of severe infectious disease, mental disorder or sleep disorder; ② No colds or other diseases in progress that may affect the experiment; ③ The subjects had regular daily life and rest habits, and their score was <7 on the Pittsburgh Sleep Quality Index (PSQI) scale; ④ No significant events leading to the possible emotional fluctuation of the subjects had occurred within 1 month before the experiment; ⑤ Subjects were required to carry out their daily activities in accordance with the standard procedures in the week before the start of the experiment, and advised to refrain from consuming stimulating beverages, carbonated beverages, tea, coffee and certain foods, as well as refraining from smoking. The study was approved by the Research Ethics Committee of Beihang University (Beijing, China). We conducted it in strict accordance with the protocol approved by the Ethics Committee, and strictly followed the requirements of the Hellenic Declaration.

Behavioral Measures

In this study, we used the Profile of Mood States Scale (POMS) to assess emotion changes (23). The advantage of this scale is that it can provide a reasonable and timely assessment of emotion changes through changes in the score. It consists of a questionnaire containing 65 items over six sections: anxiety, anger, fatigue, depression, confusion, and vitality. The scores for these items range from 0 (not at all) to 4 (very). According to the scoring principle, the scores for the 65 items are added to obtain the scores for the six sections. The top five sections form the negative emotional state assessment, and the sixth section is the positive emotional state assessment. The total score is the sum of the top five sections minus the score for the sixth section. The higher the negative emotion score, the more serious the emotional disorder, while the higher the positive score, the better the emotional state. In our study, Cronbach's alpha coefficients were satisfactory (Cronbach's alpha is 0.909).

We used the Chinese version of the PSQI, which has good psychometric properties (24). The PSQI is a self-reporting questionnaire which measures sleep quality over the previous month (25). Scores for each sleep item range from 0 (not at all) to 3 (maximum dysfunction). The scores for the sleep items are then added according to the coefficient to obtain the overall sleep quality score. We classified PSQI scores of equal to or >7 as low sleep quality.

Procedures

The design of the experiment is presented in **Figure 1**.

We experimented in the sleep laboratory of Beihang University and the magnetic resonance room of the General Hospital of the People's Liberation Army of China. All subjects participating in the experiment were divided into four batches to monitor their status more accurately and reduce the bias caused by an excessive number of subjects during the scale assessment and R-fMRI scan. During the experiment, we ensured that there were two experimenters (no fewer than one operator with a medical qualification) to guide the subjects to complete the relevant experiment contents and supervise their physical health status and experiment cooperation degree.

After preparing for the experiment in their living quarters, the subjects arrived at the laboratory at 8:00 on the first day of the experiment. They wore exercise watches to record their activity and sleep during the experiment. In the daytime of Day 1, they familiarized themselves with the experimental site and completed the evaluation. From 22:00 on Day 1 to 8:00 on Day 2, the subjects slept for at least 8 h under the supervision of the operator. After these preparations were completed, ASD started at 8:00 on Day 2 and ended at 20:00 on Day 3, during which time all subjects completed 36 h of continuous sleep deprivation. During the experiment, subjects were allowed to carry out daily activities, including playing games, reading, sitting, eating, and chatting. During the whole experiment stage, especially during the sleep deprivation stage, at least two experimenters were on hand at the same time to monitor the status of the subjects, thereby avoiding the occurrence of influencing factors such as naps during the sleep deprivation period.

We performed two R-fMRI scans during rested wakefulness (RW), and they were carried out at least 3 weeks apart to dull the effects of the exercise. The R-fMRI scan during RW used the same scan sequence as the R-fMRI scan during ASD and was performed within the same period, while the subjects completed their POMS evaluation immediately before each scan. The two scans were performed by a 3T Siemens MAGNETOM Skyra (Siemens Medical Solutions, Germany) located in the General Hospital of the People's Liberation Army. During each scan, the T1 sequence was scanned first to obtain high resolution T1-weighted anatomical images (176 images). Next, R-fMRI data collection was performed for 8 min (240 images per time). At the beginning of the scan, the subjects were asked to lie on their backs on an MRI bed with their heads fixed in a sponge and bandage. During the scan, the subjects were told to close their eyes, think of nothing and try to keep their heads and bodies still. To ensure that the subjects were awake during the scan, the operator communicated with them through a microphone before each scan to remind them to stay awake, and they were again asked if they were awake between scans of different sequences. Throughout each scan, the operator monitored the subject's body movements and other states through the viewing window. After each scan, the subjects were asked if they had been awake during the scan.

Data Processing

We used CONN toolbox software (Version 18a, Neuroimaging Informatics Tools and Resources Clearinghouse; http://www.nitrc.org/projects/conn) and SPM software (Version 12, University College London, http://www.fil.ion.ucl.ac.uk/spm) to preprocess the R-fMRI images. The two types of software are both cross-platform software based on MATLAB (Version R2018a, MathWorks, Inc. United States). First, the T1-weighted images underwent translation, segmentation and MNI normalization. Second, the first 10 volumes of the functional images were disregarded. Third, functional slice-timing correction and subject motion estimation and correction were carried out. We excluded all subjects whose mean head movements were more than 2 mm and 2°. While in the remaining subjects, the images were deleted if the head moved more than 0.5° or 0.5 mm from the adjacent images. Finally, functional indirect segmentation and normalization were carried out. In the space standardization step, the functional images were indirectly normalized to the standard space through the corresponding structural images, and the normalized bias correction is generated. The EPI template was used to normalize the structural images directly into the standard Montreal Neurological Institute (MNI) 152 standard space (voxel size of 3 × 3 × 3 mm). The full width at half maximum in smoothing was 6 mm, and the low frequency filter was 0.01–0.08 Hz. Fisher's transformation was used to normalize the distribution of variables (26, 27).

Functional Connectivity Analysis

A measure of functional connectivity between each pair of seed regions was typically calculated by region of interest to the region of interest (ROI-to-ROI) analysis. The seed regions to be studied were selected using the CONN toolbox's Automated

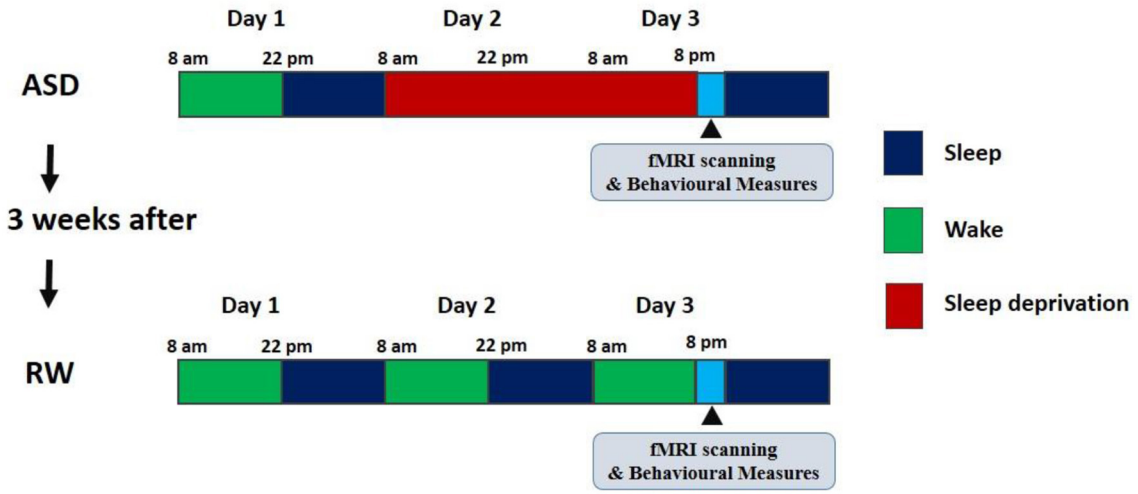

FIGURE 1 | The design of the experiment. ASD, acute sleep deprivation; RW, rested wakefulness.

Anatomical Labeling (AAL), and the location of these seed points was based on the details given by Tzourio-Mazoyer (28). A total of 90 seed regions were selected through AAL (18 seeds were removed from the cerebellar region). We then calculated the functional connectivity of the left and right thalami to the other seed regions separately for each subject. Next, Pearson correlation analysis was performed on the time series of each seed region. Finally, the Fisher Z-transformation was used to transform the correlation coefficient of each voxel and smooth the obtained data. After several corrections to the dataset, the inter-condition effect was considered to be significant at $p < 0.05$, and the group-level false discovery rate (FDR) correction p-value was <0.05.

Behavioral Measures and Correlation Analysis

SPSS (Version 21.0, IBM, Inc., USA) software was used to process the collected POMS data and other demographic data. First, descriptive statistics were carried out on each index, and a normality test was carried out on each item result and total POMS score. The measurement data was presented in the form of mean ± standard deviation. Later, a paired sample t-test was used for each section score and total POMS score before and after ASD, and $p < 0.05$ was considered statistically significant. Finally, the changes in functional connectivity coefficient between the thalamus and various brain regions ($p < 0.05$, FDR-corrected) were compared with the difference between POMS scores before and after ASD. Under the premise that the p-value was <0.05, we defined the correlation coefficients (r) ≤ 0.4 as low correlation, $0.4 < r \leq 0.6$ as moderate correlation, and $0.6 < r$ as high correlation.

RESULTS

Initial Data Quality Assessment

In the demographic description, we gave a statistical description of 28 subjects with complete data. They were all males with an average age of 24.48 ± 2.57 years, average height of 175.93 ± 5.01 cm, and average Body Mass Index (BMI) of 23.64 ± 1.73. Their PSQI scores were all <7, and their average score was 3.37 ± 1.19.

Behavioral Measures and Correlation Analysis

We used POMS to assess emotion changes before and after ASD. These scores were in line with the normal distribution, and a paired sample t-test was used to measure emotion changes. Following ASD, there were statistically significant increases in scores for anxiety ($t = 2.635$, $p = 0.014$), anger ($t = 2.066$, $p = 0.049$), fatigue ($t = 5.217$, $p < 0.001$) and confusion ($t = 4.719$, $p < 0.001$), and there was a statistically significant decrease in scores for vitality ($t = -6.464$, $p < 0.001$). The total POMS score also showed a statistically significant increase ($t = 6.215$, $p < 0.001$). In addition, decrease in depression was not statistically significant (**Table 1**).

The effect sizes of functional connectivity between the thalamus and whole-brain ROIs significantly changed ($p < 0.05$, FDR-corrected) under ASD > RW conditions are shown in **Table 2**. The ROI-to-ROI analysis demonstrated a decreased functional connectivity between the thalamus and other brain regions mainly distributed in the frontal temporal lobe, including the left middle temporal gyrus (l-MTG), right middle temporal gyrus (r-MTG), left middle temporal pole gyrus (l-MTPG), right inferior temporal gyrus (r-ITG), left orbital inferior frontal gyrus (l-OrbIFG) and left opercular inferior frontal gyrus (l-OperIFG). In contrast, an increase in functional connectivity between the left thalamus (l-Tha), left medial superior frontal gyrus (l-MSFG), right thalamus (r-Tha), left cuneus (l-Cun) and right calcarine (r-Cal) occurred during ASD. **Figure 2** shows the ROI-to-ROI functional connectivity of the left thalamus under RW, ASD

TABLE 1 | POMS statistics: comparisons between RW and ASD (paired *t*-test, $n = 28$).

Mood	RW	ASD	Mean (ASD > RW)	SD	*t*	Sig. (2 tailed)
Confusion	13.04 ± 2.40	15.00 ± 2.85	1.96	2.20	4.72	<0.001
Anxiety	14.79 ± 3.67	16.29 ± 3.73	1.50	3.01	2.64	0.014
Depression	20.61 ± 6.37	22.21 ± 7.29	1.61	5.21	1.63	0.114
Anger	16.54 ± 5.39	18.32 ± 6.63	1.79	4.57	2.07	0.049
Vitality	27.57 ± 4.39	21.04 ± 7.17	−6.54	5.35	−6.46	<0.001
Fatigue	11.14 ± 3.14	15.82 ± 5.21	4.68	4.75	5.22	<0.001
Total score	48.54 ± 20.07	66.61 ± 25.44	18.07	15.39	6.22	<0.001

TABLE 2 | ROI-to-ROI functional connectivity statistics: comparisons between RW and ASD scans (ASD > RW, paired *t*-test, $n = 28$).

Target region	Abbreviation	AAL label	MNI center	*t*	Uncorrected *p*-value	FDR-corrected *p*-value
Left thalamus	**l-Tha**	**Thalamus_L**	**−12, −18, 8**			
Left medial superior frontal gyrus	l-MSFG	Frontal_Sup_Med_L	−6, 49, 31	5.03	<0.001	0.003
Left middle temporal gyrus	l-MTG	Temporal_Mid_L	−57, −34, 30	−4.41	<0.001	0.007
Left orbital inferior frontal gyrus	l-OrbIFG	Frontal_Inf_Orb_L	−37, 31, −12	−3.81	<0.001	0.022
Right thalamus	r-Tha	Thalamus_R	12, −18, 8	3.35	0.002	0.045
Right inferior temporal gyrus	r-ITG	Temporal_Inf_R	53, −31, −22	−3.3	0.003	0.045
Left cuneus	l-Cun	Cuneus_L	−7, −80, 27	3.26	0.003	0.045
Left opercular inferior frontal gyrus	l-OperIFG	Frontal_Inf_Oper_L	−49, 13, 19	−3.19	0.004	0.045
Left middle temporal pole gyrus	l-MTPG	Temporal_Pole_Mid_L	−37, 15, −34	−3.12	0.004	0.047
Right calcarine	r-Cal	Calcarine_R	15, −73, 9	3.04	0.005	0.049
Left rectus	l-Rec	Rectus_L	−6, 37, −18	−3.01	0.006	0.049
Right thalamus	**r-Tha**	**Thalamus_R**	**12, −18, 8**			
Right middle temporal gyrus	r-MTG	Temporal_Mid_R	56, −37, −1	−3.79	0.001	0.049
Left medial superior frontal gyrus	l-MSFG	Frontal_Sup_Med_L	−6, 49, 31	−3.66	0.001	0.049

ROI, region of interest; FDR, false discovery rate; ASD, Acute sleep deprivation; RW, rested wakefulness; AAL, Automated Anatomical Labeling; MNI, Montreal Neurological Institute.

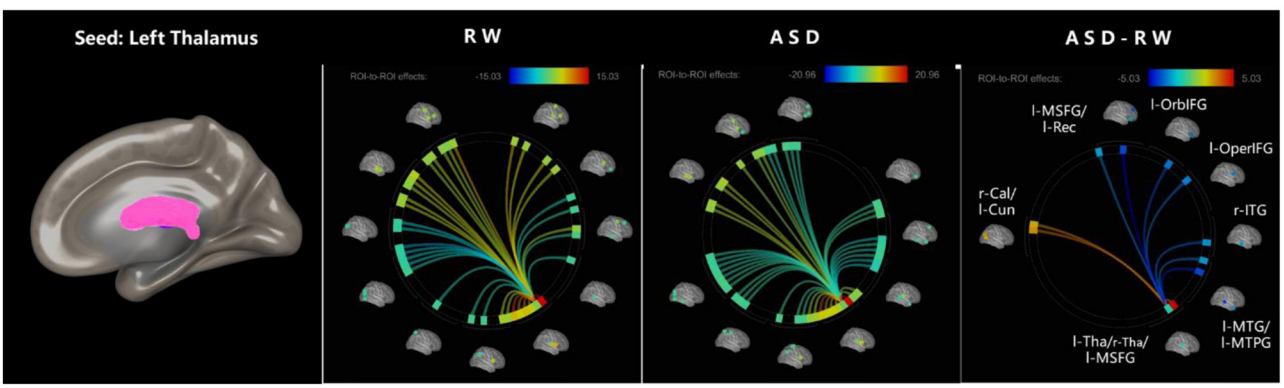

FIGURE 2 | ROI-to-ROI functional connectivity of left thalamus during RW, ASD, and ASD > RW conditions. False discovery rate-corrected ($p < 0.05$) for ROI-to-ROI tests. The functional connectivity between l-Tha and l-OperIFG, l-OrbIFG, l-MTPG, l-Rec, l-MTG, and r-ITG decreased during ASD. The functional connectivity between l-Tha and l-Cun, r-Cal, l-MSFG, and r-Tha increased during ASD. l-MSFG, left medial superior frontal gyrus; l-MTPG, left middle temporal pole gyrus; l-OrbIFG, left orbital inferior frontal gyrus; l-OperIFG, left opercular inferior frontal gyrus; l-Rec, left rectus; l-MTG, left middle temporal gyrus; r-ITG, right inferior temporal gyrus; r-Tha, right thalamus; l-Cun, left cuneus; r-Cal, right calcarine; ROI, region of interest; ASD, acute sleep deprivation; RW, rested wakefulness.

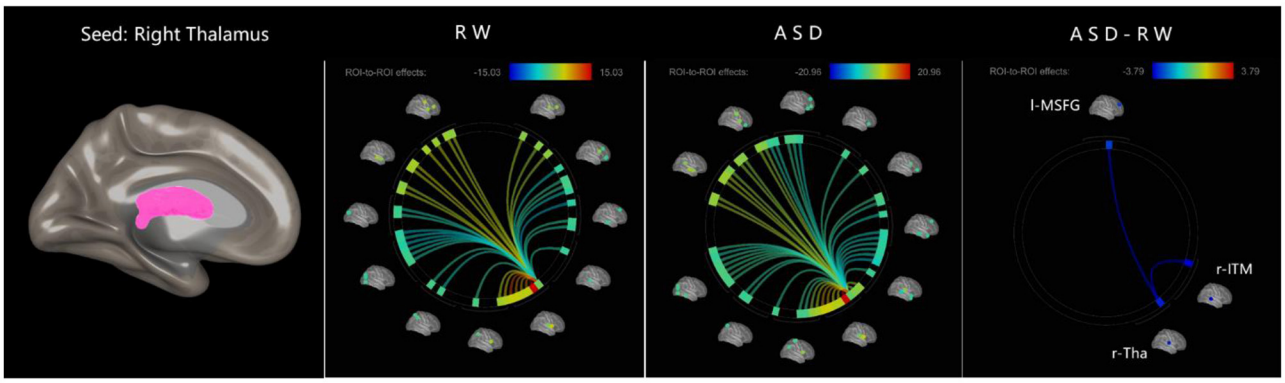

FIGURE 3 | ROI-to-ROI functional connectivity of left thalamus during RW, ASD, and ASD > RW conditions. False discovery rate-corrected ($p < 0.05$) for ROI-to-ROI tests. The functional connectivity between r-Tha and r-ITM, l-MSFG decreased decreased during ASD. r-Tha, right thalamus; r-ITM, right middle temporal gyrus; l-MSFG, left medial superior frontal gyrus; ROI, region of interest; ASD, acute sleep deprivation; RW, rested wakefulness.

and ASD > RW conditions. **Figure 3** shows the ROI-to-ROI functional connectivity of the r-Tha under the three sets of conditions.

The correlation analyses showed that emotion changes are associated with changes in the functional connections between the thalamus and parts of brain regions following ASD (**Figure 4**). The decrease in functional connectivity between the left thalamus and left orbital inferior frontal gyrus was correlated with change in emotion: r (anxiety) $= -0.446$, $p = 0.017$; r (confusion) $= -0.516$, $p = 0.005$; r (fatigue) $= -0.420$, $p = 0.026$; r (total score) $= -0.500$, $p = 0.007$. The decrease in functional connectivity between the left thalamus and left opercular inferior frontal gyrus was also correlated with emotion changes: r (anxiety) $= -0.426$, $p = 0.024$; r (total score) $= -0.396$, $p = 0.037$. However, no extra significant correlation was found between the alterations of functional connectivity and emotion changes during ASD (**Table 3**).

DISCUSSION

The role played by the thalamus in emotion changes following ASD remains unclear, although ASD can lead to emotion changes (6, 12), and the thalamus has been shown to be involved in emotion changes under CSD conditions. As an essential node in the sleep pathway, the role of the thalamus in sleep is beyond doubt (6). It is of great interest to explore the role of the thalamus in emotion changes following ASD. Our study provides preliminary evidence that the thalamus as a node is associated with emotion changes. In this study, the results of POMS confirmed that negative emotions significantly increased and positive emotions significantly decreased before and after ASD. Next, through R-fMRI analysis, significant changes were found in the functional connections between the thalamus and the brain regions that are mainly responsible for emotional processing. Finally, through correlation analysis, significant changes in the functional connections between the thalamus and related brain regions were found to be closely related to emotion changes.

Emotion Changes Following ASD

Compared to the RW state, there were significant emotion changes following ASD. The total score of the POMS scale and its five parts of anxiety, confusion, anger, vitality and fatigue all changed significantly. This is consistent with other relevant studies (10, 11, 29–31). Furthermore, these results largely support those of Short and Louca (32) and Babson et al. (31), which show that ASD is sensitive to different emotional deficits under different emotional states, and has a more significant impact on confusion, energy and fatigue than on depression, anxiety and anger (31, 32). Moderate sleep deprivation aggravates confusion, vigor and fatigue, and emotional states such as anger and anxiety often worsen with more severe sleep deprivation, especially after complete sleep deprivation (31, 32). We agree with Mischel's reasoning about this changing trend and argue that people with different emotional states are sensitive to sleep deprivation differently. It's important to note that our study found no significant change in depressive mood following ASD. This is consistent with relevant studies to a certain extent, as ASD is an effective treatment for depression, with well-documented efficacy around 50% (33, 34). This may explain the absence of significant changes in depression in healthy subjects following ASD. Besides, our subjects within each group knew each other and did not undergo high-intensity experimental content, which may have reduced the degree of specific negative emotion changes such as depression during the experiment.

Changes in Brain Functional Connectivity

The functional connectivity between the thalamus and other brain regions was significantly changed following ASD, and these regions are mainly located in the frontal and parietal cortex which are involved in almost all functions related to emotion (5–7, 35). We observed significantly changed functional connections between the thalamus and the left inferior frontal gyrus, left middle temporal gyrus, right middle temporal gyrus, right inferior temporal gyrus and left medial superior frontal gyrus, all of which are involved in emotional function to some extent. Actually, almost the entire brain network is

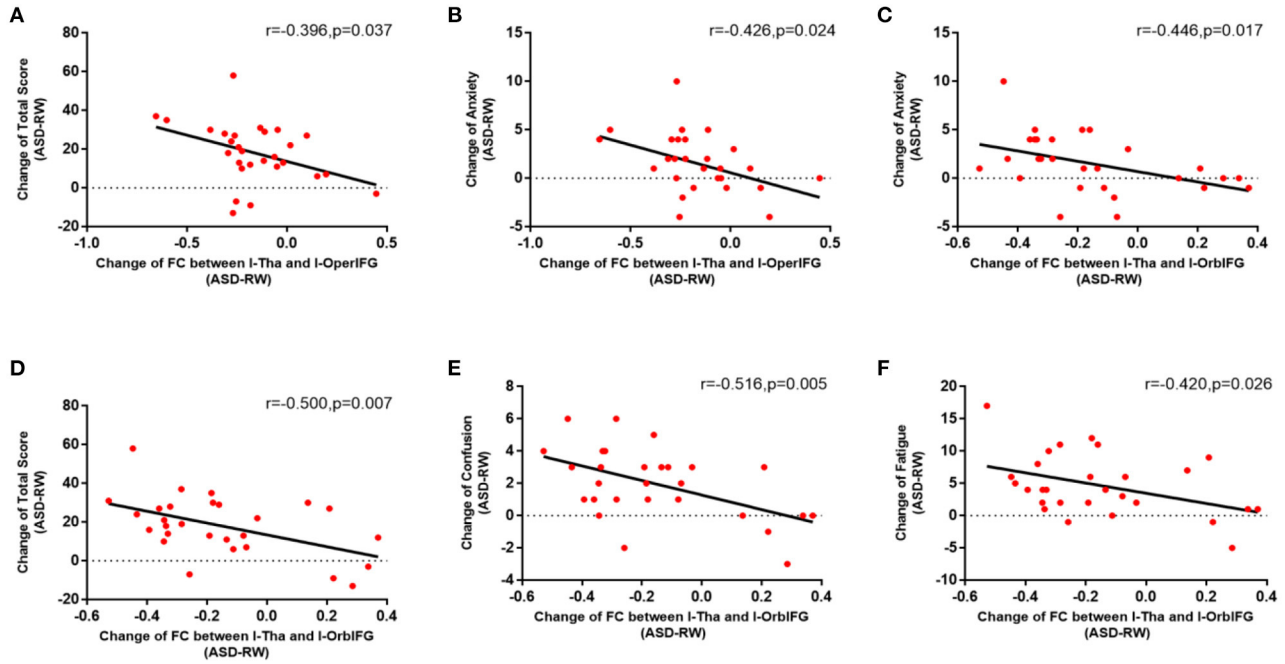

FIGURE 4 | Relation of functional connectivity between left thalamus and other brain regions to emotion changes following acute sleep deprivation (ASD > RW, $n = 28$). The decrease in functional connectivity between l-Tha and l-OperIFG **(A)**, between l-Tha and l-OrbIFG **(D)** was significantly negatively correlated with the Change of total score of the POMS. The decrease in functional connectivity between l-Tha and l-OperIFG **(B)**, between l-Tha and l-OrbIFG **(C)** was significantly negatively correlated with the change of anxiety. The decrease in functional connectivity between l-Tha and l-OrbIFG **(E,F)** was significantly negatively correlated with the change of confusion and fatigue. FC, functional connectivity; ASD, acute sleep deprivation; RW, rested wakefulness; l-Tha, left thalamus; l-OperIFG, left opercular inferior frontal gyrus; l-OrbIFG, left orbital inferior frontal gyrus.

TABLE 3 | Correlation between changes in functional connectivity and changes in POMS ($n = 28$).

Mood	Brain regions (changed to the left thalamus)									
	l-MSFG	l-MTPG	l-OrbIFG	l-OperIFG	l-Rec	l-MTG	r-ITG	r-Tha	l-Cun	r-Cal
Confusion	−0.117	0.041	−0.516*	−0.116	−0.158	0.022	0.031	0.181	0.335	0.109
Anxiety	−0.253	0.114	−0.446*	−0.426*	−0.123	−0.277	−0.26	0.278	0.368	0.052
Depression	−0.115	−0.096	−0.199	−0.315	−0.097	−0.252	−0.029	0.292	0.385*	0.064
Anger	−0.114	0.161	−0.172	−0.242	0.12	−0.206	0.039	0.271	0.114	0.017
Vitality	0.285	−0.07	0.261	0.15	0.245	0.009	−0.067	−0.137	0.208	−0.147
Fatigue	−0.176	−0.163	−0.420*	−0.21	−0.257	−0.249	−0.143	0.159	0.192	0.207
Total Score	−0.292	0.018	−0.500*	−0.396**	−0.208	−0.277	−0.066	0.356	0.271	0.167

*Pearson correlation analysis: *$p < 0.05$ and $0.4 < r \leq 0.6$, moderate correlation; **$p < 0.05$ and $r \leq 0.4$, low correlation.*
l-MSFG, left medial superior frontal gyrus; l-MTPG, left middle temporal pole gyrus; l-OrbIFG, left orbital inferior frontal gyrus; l-OperIFG, left opercular inferior frontal gyrus; l-Rec, left rectus; l-MTG, left middle temporal gyrus; r-ITG, right inferior temporal gyrus; r-Tha, right thalamus; l-Cun, left cuneus; r-Cal, right calcarine.

involved in emotional functions (36), and sleep deprivation can reliably trigger changes in negative emotional processes including irritability, anxiety, aggression, and mood swings (6). Meanwhile, although the thalamus is an essential node of wakefulness switching in the sleep-wake pathway, we noticed no significant change in the functional connections between the thalamus and the amygdala, an emotion-processing region of the limbic system which is susceptible to ASD (6, 37). These changes in functional connections between the thalamus and other brain regions associated with emotion changes strongly suggest that the thalamus may be involved in emotion changes to some extent. This may support the idea that in emotional experience and the perception of a set of discrete categories of emotion, a group of interactive brain regions is usually involved in emotional and non-emotional basic psychological operations (37).

Relation of Altered Functional Connectivity to Emotion Changes

Our study of the relation of altered functional connectivity to emotion changes further confirms that the thalamus is

involved in emotion changes following ASD. It was found that the functional connections between the left thalamus and left orbital inferior frontal gyrus were negatively correlated with the total POMS score and confusion, anxiety and fatigue. The orbital frontal gyrus consists of medial ventral parts of the superior, middle and inferior frontal gyrus. It processes emotional responses to internal cues and modulates emotions and rewards in the decision-making process. In particular, the left orbital inferior frontal gyrus is closely related to emotion changes, and the impairment of the orbital frontal regulation of limbic emotional processing is considered the cause of the bipolar disorder (38, 39).

Our study also observed that the functional connections between the left thalamus and left opercular inferior frontal gyrus were negatively correlated with the total POMS score and anxiety (**Table 3**). The left opercular inferior frontal gyrus is an essential area for language processing (40), and the relatively few relevant studies have pointed out that there is a specific correlation between the left opercular inferior frontal gyrus and social anxiety disorder (15, 41–43).

The above results show that the functional connections between the left thalamus and left inferior frontal gyrus are correlated with emotion changes following ASD, especially anxiety (**Figure 4**). The inferior frontal gyrus is comprised of the orbital inferior frontal gyrus, triangular inferior frontal gyrus and opercular inferior frontal gyrus (44). The study found significant changes in frontal lobe function during sleep deprivation (45), and certain studies have suggested that functional changes in the left inferior frontal gyrus during sleep deprivation are associated with anxiety, depression and mood swings (7, 43, 46, 47). Given the various functions of the left inferior frontal gyrus, it is difficult to distinguish whether it is the responsible center or relay station of emotion changes according to the existing research, but it is clear that the left inferior frontal gyrus is an important hub of the emotional pathways.

The left thalamus engages in a variety of emotion changes presented in many diseases such as anxiety disorder, bipolar disorder, posttraumatic stress disorder, major depressive disorder, and hyperalexithymia (8, 48). Patients with high stress are specifically associated with lesions in the left thalamus (49) since both the left thalamus and left inferior frontal gyrus are involved in a variety of emotion changes, and the inferior frontal gyrus interacts closely with the thalamic nuclei (44). Furthermore, studies have suggested that CSD is characterized by structural and functional changes between the thalamus and left inferior frontal gyrus (50–52). Meanwhile, the functional connections between the thalamus and frontal lobe seem sensitive to mild sleep-wake changes (53) which are similar to those observed following ASD in our study. Most importantly, our experiment shows that the functional connections between the left thalamus and left inferior frontal gyrus are strongly associated with emotion changes such as anxiety. Taken together, there is sufficient preliminary evidence to demonstrate the involvement of the thalamus in emotion changes induced by ASD.

Furthermore, although the functional connections between the bilateral thalamus and medial superior frontal gyrus decreased, there was no clear correlation between this decrease and emotion changes. The medial superior frontal gyrus, one of the most important brain regions in the emotional network, showed altered functional connections with the bilateral thalamus during ASD, but this did not seem to be the leading cause of the emotion changes. Meanwhile, our results showed that the functional connections between the thalamus and other brain regions mainly responsible for emotion were not significantly changed following ASD, which further suggests that the brain regions affected by ASD and those affected by CSD may not be entirely consistent.

We further speculate that the emotion changes under ASD related to the left inferior frontal gyrus and thalamus are not entirely consistent with the traditional emotional network. The thalamus has been observed to participate in a variety of functional networks that have different response patterns (54). This suggests that functional tissues allow spatially overlapping networks of resting states of the brain, facilitating the description of various interpersonal relationships between overlapping regions and different functional systems in other regions of the brain (54). This partly explains the decreased functional connections between the left thalamus and left inferior frontal gyrus associated with emotion changes after sleep deprivation. It is also possible that there is a network mechanism for new emotion changes in the context of ASD.

In summary, our study finds that functional changes in the thalamus following 36 h of total ASD are associated with emotion changes. The changes in functional connection between the left thalamus and left inferior frontal gyrus were negatively correlated with emotion changes, and the thalamic-related emotion regulation circuit was affected. This means that the left thalamus plays a vital role in emotion changes following ASD.

LIMITATIONS OF THE STUDY

First, the subjects of our study were all young male men limited to the experimental conditions, and literature has pointed out that gender may have different effects on emotional state (e.g., depression) during sleep deprivation (55). Gender and age may affect the accuracy of the experimental conclusion, we should take the gender differences into consideration in future fMRI studies, especially the treatment of brain-related diseases. We will build on the present study and refine our study in future studies to cover left-handedness and sleep deprivation in women. Second, the thalamus is a big ROI in AAL, and calculations based on this seed point may be general. However, for the selection of ROI in AAL, we mainly considered that this template was widely used, especially in the previous thalamic research articles. Therefore, we chose this conservative seed point in this exploratory study. Furthermore, we will study the function of the thalamus by using a more detailed method

in further studies to further improve this conservative but robust conclusion.

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 Research Ethics Committee of Beihang University (Beijing, China; BM20180040). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

B-zL contributed to performing the experiments, processing the data and drafting the paper. YCa contributed to processing the data and drafting the paper. YZ and YCh contributed to performing the experiments and acquiring the data. Y-cS, Y-hG, and XZ are the guarantors of the study. All authors made substantial contributions to this work and approved it for publication.

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Compromised Dynamic Cerebral Autoregulation in Patients With Central Disorders of Hypersomnolence

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Objective: We aimed to investigate the dynamic cerebral autoregulation (dCA) in patients with central disorders of hypersomnolence during wakefulness.

Methods: Thirty-six patients with central disorders of hypersomnolence were divided into three groups according to polysomnography and multiple sleep latency test results: the idiopathic hypersomnia group (IH), narcolepsy type 1 without rapid-eye-movement sleep behavior disorder group (NT1-RBD), and narcolepsy type 1 with rapid-eye-movement sleep behavior disorder group (NT1 + RBD), with 12 patients in each group. Twelve sex- and age-matched healthy controls were recruited. We assessed the Epworth sleepiness scale (ESS) and dCA of all subjects. dCA was assessed by analyzing the phase difference (PD) using transfer function analysis. The ESS and dCA were analyzed before and after standardized treatment in 24 patients with narcolepsy type 1.

Results: The overall PD of the IH, NT1-RBD, and NT1 + RBD groups were lower than that of the control group ($P < 0.001$). There were no significant differences between the overall PD of the NT1-RBD and NT1 + RBD group ($P > 0.05$). The ESS scores decreased and the overall PD increased after treatment in 24 patients with narcolepsy type 1 ($P < 0.001$). Multivariable analysis showed that mean sleep latency in multiple sleep latency test was independently associated with impaired overall PD ($P < 0.05$).

Conclusions: The dCA is impaired in patients with central disorders of hypersomnolence. The impairment of dCA occurs irrespective of NT1-RBD/+RBD. The ESS score and dCA improved in patients with narcolepsy type 1 after medication treatment. The mean sleep latency in multiple sleep latency test was independently associated with impaired dCA.

Clinical Trial Registration: www.ClinicalTrials.gov, identifier: NCT02752139.

Keywords: central disorders of hypersomnolence, narcolepsy type 1, idiopathic hypersomnia, rapid-eye-movement sleep behavior disorder, dynamic cerebral autoregulation

INTRODUCTION

The central disorders of hypersomnolence include narcolepsy type 1 (NT1), narcolepsy type 2 (NT2), and idiopathic hypersomnia (IH) (1). Studies have demonstrated a decreased cerebral blood flow (CBF) in patients with NT1 and IH (2, 3), which implies that cerebral autoregulation (CA) function may play an important role in the central disorders of hypersomnolence. CA is a physiological mechanism of the brain that maintains sufficient CBF despite changes in the blood or cerebral perfusion pressure. Dynamic cerebral autoregulation (dCA) can respond to real-time changes in the blood pressure within seconds, allowing continuous measurement of CA and a wave by wave analysis of hemodynamics (4). Many diseases may impair dCA, including cerebrovascular diseases, traumatic brain injury and Alzheimer's disease (5, 6). The dCA mechanism is complex; it includes myogenic, metabolic, endothelial, and neurogenic mechanisms, and neurogenic including the monoaminergic and cholinergic mechanisms (7). Studies have found the monoaminergic mechanism including 5-hydroxytryptamine and norepinephrine to be implicated in the central disorders of hypersomnolence (8, 9), which are vasoactive substances and likely affect the dCA (10, 11).

Rapid-eye-movement sleep behavior disorder (RBD) affects 30–63% of NT1 patients (12). Idiopathic RBD exhibits decreased cerebral perfusion and dCA (13, 14); however, there are currently no studies on RBD and dCA in NT1 patients. We hypothesize that dCA is impaired in patients with central disorders of hypersomnolence. We obtained dCA data from patients with IH and NT1 and investigated the relationship between central disorders of hypersomnolence and dCA.

METHODS

The study was approved by the ethics committee of the First Hospital of Jilin University and followed the guidelines of the Declaration of Helsinki (1964). Written informed consent was obtained from all the participants or their relevant guardians.

Subjects

Patients with central disorders of hypersomnolence, that underwent the polysomnography (PSG) and multiple sleep latency tests (MSLT), were recruited from the Department of Neurology, First Hospital of Jilin University, from March 2018 to March 2019. A total of 36 patients were enrolled with central disorders of hypersomnolence [12 patients with IH, 12 with NT1 without RBD group (NT1-RBD), and 12 with NT1 with RBD group (NT1 + RBD), respectively]. Twelve age- and sex-matched healthy controls were also recruited from the same region, based on their PSG results.

Inclusion Criteria

The inclusion criteria for the different groups were as follows: (1) Control group: age- and sex-matched healthy volunteers without sleep disorder in PSG; (2) NT1-RBD and NT1 + RBD groups: subjective sleepiness, cataplexy, mean sleep latency $\leq$8 min, and $\geq$2 sleep-onset rapid-eye-movement (REM) periods (SOREMP) on MSLT or one SOREMP on the preceding night PSG coupled with one SOREMP on the MSLT. The diagnostic criteria of RBD was based on the International Classification of Sleep Disorders 3rd edition; (3) IH group: subjective sleepiness, absence of cataplexy, mean sleep latency $\leq$8 min, and with one time or without SOREMP (including no SOREMP on the PSG from the preceding night); (4) the age cut-off value being 15 to 65; and (5) good bilateral temporal window penetration.

Exclusion Criteria

The exclusion criteria were as follow: presence of (1) sleep-related breathing disorders, circadian rhythm disorders, and other causes of disturbed nocturnal sleep; (2) history of secondary daytime sleepiness, epilepsy, mental illness, or drug abuse; (3) arrhythmia, hyperthyroidism, and other hemodynamic factors; (4) intracranial and extracranial vascular stenosis or occlusion diagnosed by vascular ultrasound; and (5) not cooperating with the questionnaire survey.

Collection of Clinical Data

All subjects underwent a comprehensive collection of general and clinical data, including age, sex, medical history, Epworth sleeping scale (ESS) evaluation, neurological examination, and head magnetic resonance imaging.

Polysomnography

All the subjects were monitored for at least 8 h in the sleep center of our hospital using PSG (Compumedics, Australia). The PSG results were analyzed by professional sleep technicians, that had PSG technologist certification, by referring to the revised interpretation criteria of sleep stages and related events issued by the American Academy of Sleep Medicine version 2.1.

Multiple Sleep Latency Tests

After PSG recording, the central electroencephalogram (C3-A2, C4-A1) and occipital electroencephalogram (O1-A2, O2-A1), left and right electrooculogram, electromyography, and electrocardiography were retained. The first nap was performed 2–3 h after the PSG examination. Afterward, 4–5 naps were taken at intervals of 2 h. Activities were avoided 15 min before each test. Sleep was avoided during the 2 h interval. A total of 15 min were recorded after falling asleep, including the 20 min before falling asleep. If only one SOREMP was recorded in the first four naps, an additional 5th nap was also recorded.

Dynamic Cerebral Autoregulation Measurement

The participants were instructed to avoid alcohol, nicotine, and caffeine intake, and exercise for at least 12 h before the measurement. The measurement was performed in a quiet, dedicated research laboratory. First, the subjects had their baseline arterial blood pressure (Omron 711) and heart rates measured (15). Then beat-to-beat arterial blood pressure and continuous bilateral middle cerebral artery blood flow velocity were recorded for 10 min. The measurement data were then stored for further dynamic cerebral autoregulation examination analysis (16).

TABLE 1 | Clinical characteristics in the patients with IH, NT1-RBD, NT1 + RBD, and controls.

	Controls ($n = 12$)	IH ($n = 12$)	NT1-RBD ($n = 12$)	NT1 + RBD ($n = 12$)	*P*-value
Gender (male/female)	8/4	8/4	7/5	8/4	0.965
Age (years)	31.9 ± 7.9	39.8 ± 13.9	29.4 ± 10.3	34.9 ± 13.8	0.177
Mean ABP (mmHg)	83.3 ± 6.1	87.9 ± 5.0	84.7 ± 5.8	83.3 ± 5.3	0.061
Heart rate (bmp)	71.5 ± 6.2	70.9 ± 10.0	69.8 ± 7.7	70.2 ± 5.6	0.944
End-tidal CO_2 (mmHg)	36.6 ± 3.6	36.7 ± 4.0	37.0 ± 3.9	36.6 ± 2.6	0.990

IH, idiopathic hypersomnia; NT1-RBD, narcolepsy type 1 without rapid-eye-movement sleep behavior disorder; NT1 + RBD, narcolepsy type 1 with rapid-eye-movement sleep behavior disorder; ABP, arterial blood pressure.

Analysis of Dynamic Cerebral Autoregulation

Recorded data were processed using the MATLAB software (Math Works, Natick, MA, USA). The data analysis of dCA was performed using the transfer function analysis (TFA) (15). TFA between the arterial blood pressure and cerebral blood flow velocity was calculated as the quotient of the cross-spectrum of the two signals and the auto spectrum of arterial blood pressure in the low-frequency domain (0.06–0.12 Hz) to obtain the frequency-dependent estimates of phase difference (PD), where the derived parameters are considered to be the most relevant to autoregulation hemodynamics (17). A decreased PD represents impaired dCA. Coherence was calculated to estimate the reliability of the relationship between the two signals at the frequency domain, and the later statistical analysis was performed only if the coherence of the parameters was >0.5 (18).

Statistical Analysis

The SPSS 23.0 software was used for statistical analysis. The Shapiro-Wilk test was used to assess the normal distribution of continuous variables. Measurement data with a normal distribution [including ESS, PD, age, mean arterial blood pressure (ABP), heart rate, and end-tidal CO_2] were expressed as mean and standard deviation whereas data with skewed distribution were expressed as median (interquartile range). Categorical data (gender) were expressed as absolute values. Paired *t*-test was used to compare the differences between the two paired samples; ESS and PD in patients with NT1, before and after treatment. One-way ANOVA or Kruskal-Willis H test was used to compare the differences between the multiple groups of independent samples (ESS and PD of IH, NT1-RBD, NT1 + RBD, and control groups) based on the data distribution. Univariate and multivariate linear regression were used to assess the association of PD and clinical parameters including the total sleep time (TST), mean sleep latency in MSLT, sleep efficiency, percent of stage 1 non-REM (NREM) time in TST (stage N1), percent of stage 2 NREM time in TST (stage N2), percent of stage 3 NREM time in TST (stage N3), percent of REM sleep time in TST (REM sleep), and arousal index. In the *post-hoc* analysis, the Bonferroni method was used to calculate the adjusted *P*-value. *P*-values below 0.05 were considered statistically significant.

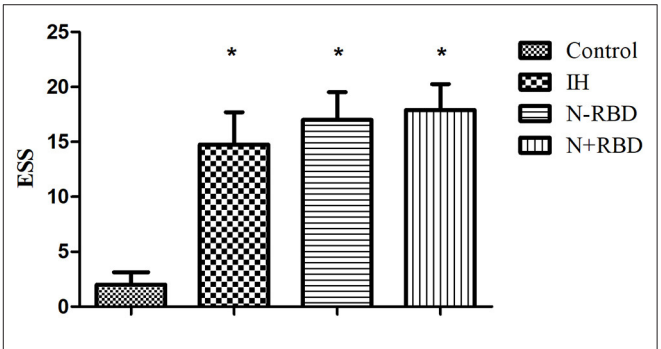

FIGURE 1 | ESS scores in the patients with IH, NT1-RBD, NT1 + RBD, and controls. *Different from controls ($P < 0.05$). ESS, Epworth sleepiness scale.

RESULTS

Baseline Characteristics

In total, 48 participants were enrolled in this study, including 12 IH patients, 12 NT1-RBD patients, 12 NT1 + RBD patients, and 12 controls. There are no significant differences in gender, age, mean ABP, heart rate, and end-tidal CO_2 among all the groups. The clinical characteristics of the participants are described in **Table 1**.

ESS Score

ESS was used to assess the degree of sleepiness. The ESS scores of patients in the IH, NT1-RBD, and NT1 + RBD groups (14.8 ± 3.0, 17.0 ± 2.5, 17.9 ± 2.4) were higher than those of the control group (2.1 ± 1.1, $P < 0.001$) (**Figure 1**).

dCA Parameters

The overall PD of patients in the IH, NT1-RBD, and NT1 + RBD groups (34.36 ± 6.51, 35.96 ± 8.99, 32.90 ± 9.98°) was significantly lower than that of the control group (52.98 ± 6.33°, $P < 0.001$) (**Figure 2**); however, there were no significant differences between the overall PD of the NT1-RBD and NT1 + RBD group (35.96 ± 8.99 vs. 32.90 ± 9.98°, $P > 0.05$).

ESS and dCA Parameters of NT1 Before and After Treatment

Among 24 patients with NT1, the ESS and dCA were examined after 1-month treatment with methylphenidate (18 mg, once a day in the morning) and venlafaxine (75 mg, once a day in the

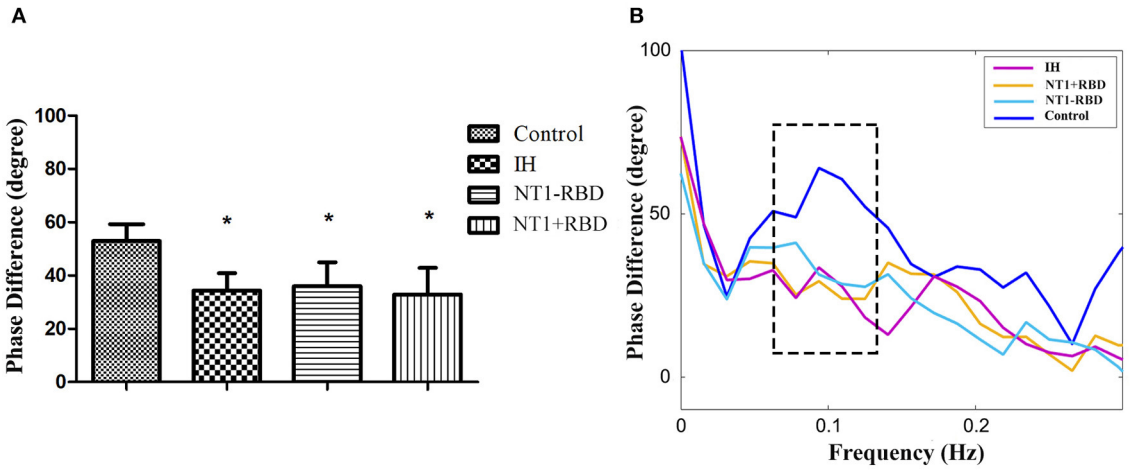

FIGURE 2 | The autoregulatory parameter and statistical distributions in the patients with IH, NT1-RBD, NT1 + RBD, and controls. Statistical distributions of overall phase difference **(A)** and its transfer function **(B)** in each group. *Difference of overall phase difference in patients with IH, NT1-RBD, NT1 + RBD, and controls ($P < 0.05$).

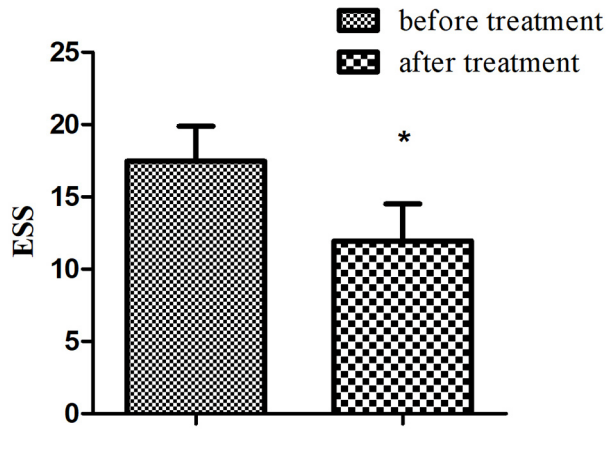

FIGURE 3 | Comparison of ESS in narcolepsy type 1 group before and after treatment. *Represents statistical difference ($P < 0.05$).

morning). The ESS score decreased (11.96 ± 2.55 vs. 17.46 ± 2.43, $P < 0.001$) (**Figure 3**) and the overall PD increased after the treatment (47.37 ± 9.31 vs. 34.43 ± 9.42°, $P < 0.001$) (**Figure 4**).

Univariable and Multivariable Analyses

The clinical parameters used in the univariable and multivariable analysis are shown in **Table 2**. In the univariable model, the overall PD correlated with mean sleep latency in MSLT ($P < 0.001$), sleep efficiency ($P < 0.05$), stage N1 ($P < 0.05$), and arousal index ($P < 0.05$). The multivariable model included mean sleep latency in MSLT, sleep efficiency, stage N1, stage N2, stage N3, and arousal index. Mean sleep latency in MSLT ($P < 0.05$) was an independent factor that influenced the overall PD (**Table 2**).

DISCUSSION

We found the cerebral autoregulatory parameter compromised in patients with central disorders of hypersomnolence. There were no significant differences in the degree of impairment between the NT1-RBD and NT1 + RBD patients. The ESS score and dCA improved after medication for the NT1 patients. The mean sleep latency in MSLT was independently associated with impaired dCA.

Studies have found abnormal CA in NT1 patients, evident by hypoperfusion of the hypothalamus, thalamus, prefrontal cortex, hippocampus, and cingulate gyri by single-photon emission computed tomography (2, 19), and abnormal CA is most likely implicated in the progression of neurological symptoms (such as cataplexy and sleep paralysis). The pathogenesis of compromised dCA in NT1 patients is the deficiency of hypocretin and monoaminergic neurons (9, 20). Hypocretin is involved in REM sleep and motor regulation through its effects on monoaminergic cells, including dopamine (DA), norepinephrine (NA), 5-hydroxytryptamine (5-HT), and other neurons (9, 21). NA is a sleep autonomic neuromodulating transmitter, which is an important factor for dCA (10). 5-HT is a vasoactive substance and has a potential role in dCA (11). In addition, hypocretin deficiency and a lower concentration of monoaminergic neurotransmitters lead to decreased activated projection to the basal forebrain and tuberomammillary nucleus; they are responsible for the maintenance of cortical arousal (22). Furthermore, the hypocretin neurons are located in the lateral hypothalamus and around the fornix, which projects to the regulatory centers of several autonomic nerves, including the autonomic neurons in the periaqueductal gray matter, the nucleus tractus solitarius, the nucleus ambiguous, the dorsal vagal nucleus, and the intermediolateral column of the spinal cord. Reduced or absent hypocretin levels in the inferior colliculus may reduce sympathetic excitability in NT1 patients

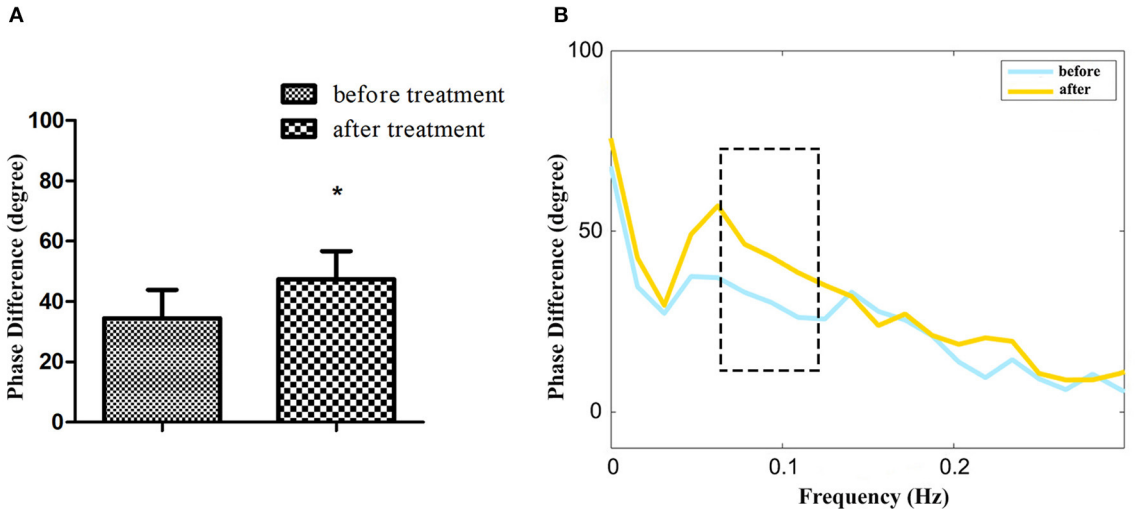

FIGURE 4 | The autoregulatory parameter and statistical distributions in narcolepsy type 1 group before and after treatment. Statistical distributions of phase difference **(A)** and its transfer function **(B)** in narcolepsy type 1 group before and after treatment. *Represents statistical difference ($P < 0.05$).

TABLE 2 | Univariable and multivariable analysis of polysomnography parameters associated with the overall phase difference.

Factors	Overall phase difference (°)			
	Univariable analysis		Multivariable analysis	
	β	*P*	*β*	*P*
TST (min)	0.107	0.555		
Mean sleep latency in MSLT (min)	0.647	<0.001[ab]	0.470	0.016[bc]
Sleep efficiency (%)	0.416	0.016[ab]	0.059	0.785
Stage N1 (%)	−0.559	0.001[ab]	−0.515	0.128
Stage N2 (%)	0.330	0.060[a]	−0.168	0.511
Stage N3 (%)	0.298	0.092[a]	0.246	0.379
REM sleep (%)	0.238	0.183		
Arousal index	−0.376	0.031[ab]	−0.002	0.993

TST, total sleep time; MSLT, multiple sleep latency test; REM, rapid eye movement; stage N1, percent of stage 1 non-REM (NREM) time in TST; stage N2, percent of stage 2 NREM time in TST; stage N3, percent of stage 3 NREM time in TST; REM sleep, percent of REM sleep time in TST.

[a]*Nominally significant values ($P < 0.1$) included in the multivariable model.*

[b]*P-value < 0.05 (statistically different).*

[c]*Independent factor that influences overall phase difference.*

(23). Our study showed that the dCA was impaired in NT1 patients, which may be related to hypocretin and monoamine neurotransmitter deficiency.

Narcolepsy is the second cause of RBD. Knudsen et al. found hypocretin deficiency to be independently associated with the prevalence of RBD outcomes (symptoms and muscle activity) during REM sleep in narcolepsy (24), which may explain the absence of a significant difference between the overall PD in the NT1-RBD and NT1 + RBD group; they may share the same mechanism. RBD sometimes can be the heralding symptom of NT1, forerunning the occurrence of cataplexy (25).

The pathological mechanism of IH is currently unclear. Studies have shown a normal concentration of hypocretin in the cerebrospinal fluid of IH patients; however, with low levels of DA, NA and 5-HT metabolites (8, 26, 27). In addition, IH patients have autonomic symptoms (cold extremities, palpitations, and fainting episodes) (27). We hypothesize that the impaired dCA in IH patients may be related to the lower concentration of monoaminergic neurotransmitters.

Drug therapy for narcolepsy in this study included methylphenidate and venlafaxine. Methylphenidate is a non-competitive DA reuptake blocker, which inhibits 5-HT and NA to a certain extent. Venlafaxine is a 5-HT-NA reuptake inhibitor that increases the concentration of 5-HT and NA. After 1-month treatment with the two drugs, the symptoms of EDS and the dCA improved in patients with narcolepsy, which may be related to the increased blood concentration of 5-HT and NA, and is consistent with previous reports (28, 29).

The MSLT is an objective test that measures the tendency to fall asleep under controlled conditions. It is based on the notion that sleep latency reflects the underlying physiological sleepiness (30). Since the mean sleep latency is shortened in IH and NT1 patients on MSLT, we found the mean sleep latency in MSLT to be independently associated with the impaired dCA. The potential mechanisms are still unclear. We hypothesize the 5-HT and NA levels to be a potential mechanism that explains the underlying relationship between the mean sleep latency and the dCA; larger sample size is required to confirm this result.

Our study shows that dCA is impaired in patients with central disorders of hypersomnolence, which may involve the monoaminergic mechanism; the relationship with hypocretin remains to be explored. The compromised dCA in patients with central disorders of hypersomnolence can explain and predict the clinical symptoms, and provide a new dynamic evaluating method for central disorders of hypersomnolence.

This study has some limitations. First, we did not examine the hypocretin of cerebrospinal fluid. Second, we did not detect the monoaminergic neurotransmitters in the participants' serum. Third, the sample size of this study is small, which may limit the analysis.

CONCLUSION

The dCA is impaired in patients with central disorders of hypersomnolence. The dCA impairment occurs irrespective of NT1-RBD/+RBD. The ESS score and dCA improved in patients with NT1 after medication. The mean sleep latency in MSLT was independently associated with impaired dCA.

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 First Hospital of Jilin University. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

FD, YZ, and ZG wrote the manuscript. RZ conducted the data acquisition and data analysis. QT prepared the figures. YL analyzed the PSG. ZW and YY managed the study and edited the final manuscript. All authors contributed to the article and approved the submitted version.

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Circadian Rhythm Disorders and Corresponding Functional Brain Abnormalities in Young Female Nurses: A Preliminary Study

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Objective: Shift work is associated with a decrease in melatonin level and perturbation of the circadian rhythm; however, it is unknown if these lead to functional brain changes. In this study, we investigated whether circadian rhythm disorders caused by shift work are related to changes in brain functional connectivity (FC) and regional homogeneity (ReHo) using whole-brain resting-state functional magnetic resonance imaging (fMRI).

Methods: This prospective case-control study included nine female night shift nurses and nine age-matched female day work nurses with normal sleep rhythms. To assess sleep quality and mood, participants were asked to complete questionnaires. Serum melatonin and cortisol levels were measured. ReHo of whole-brain resting-state function and seed-based FC of the bilateral hypothalamus were compared between groups. Variables that differed significantly between groups were used to examine the association between questionnaire scores and hormone levels and fMRI data.

Results: The night shift nurses had significantly lower sleep quality and melatonin levels; lower ReHo activation in the bilateral cerebellar hemisphere and higher ReHo in the bilateral occipital lobe and left parietal lobe; and higher FC from the hypothalamus to the right cingulate gyrus, right putamen, and vermis than did the day shift nurses. Activation of the right cerebellar hemisphere left superior parietal gyrus, and the right superior occipital gyrus was correlated with sleep quality scores. Moreover, activation of the right cerebellar hemisphere ($r = 0.583$, $P = 0.011$) was correlated with melatonin levels, and higher sleepiness scores were associated with stronger FC between the hypothalamus and vermis ($r = 0.501$, $P = 0.034$).

Conclusions: Circadian rhythm disorder caused by night shift work can lead to a decrease in sleep quality and melatonin level, as well as a series of changes in brain FC and ReHo.

Keywords: melatonin, circadian rhythm, resting functional magnetic resonance imaging, regional homogeneity, functional connectivity

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INTRODUCTION

The conflict between the social demands of modern life and endogenous rhythms has resulted in circadian rhythm disorders and a host of mental and physical health issues. These issues are typified by shift work, which is underscored by an imbalance between social work/rest time and internal circadian rhythms. With industrialization, travel across time zones, use of electronic products, and light exposure of modern long-term indoor life, individuals are forced to ignore or inhibit the natural circadian rhythm. For example, the use of personal electronic devices rich in blue light at night can delay circadian rhythm and the start of sleep, inhibit melatonin secretion, and increase morning sleepiness (1). In addition, individual sensitivity to light circadian rhythm also demonstrates individual differences. Therefore, a more in-depth understanding of circadian rhythm disorders, as well as possible hormonal changes and brain function changes, will facilitate health management and treatment.

Effects of sleep and circadian rhythm disorders on health and cognitive ability are well-documented. Indeed, prolonged night shift work is associated with an increased risk of cardiovascular disease (2). Shift work is associated with increased secretion of cortisol and catecholamines (3, 4) and decreased secretion of melatonin (5), which may result in abnormal hormone secretion rhythms. A decline in melatonin levels in the morning is related to activation of the prefrontal cortex, indicating that changes in melatonin levels in the morning may be related to cortical alertness, attention, and executive ability (6). Furthermore, a decrease in attention and alertness as well as significant changes in brain function, occur after sleep deprivation (7). Additionally, insomnia and anxiety increase significantly, and cerebral perfusion changes occur among shift workers (8). This previous evidence demonstrates that the abnormality of circadian rhythm could not only lead to hormone secretion disorders, attention deficits, and sleep disorders but also affect brain function. Differences in circadian phenotype between "owls" and "larks" are associated with differences in brain functional connectivity (FC), especially in the default mode network (DMN), which is related to attention and subjective sleep disorders (9). One study compared seed connectivity of the suprachiasmatic nucleus using resting-state fMRI to determine the possible mechanisms of circadian rhythm disorders in patients with delirium and revealed that FC from the suprachiasmatic nucleus to the dorsal anterior cingulate cortex increased, whereas FC to the posterior cingulate gyrus, parahippocampal gyrus, cerebellum, and thalamus decreased (10). These findings suggest that resting-state fMRI is a useful tool for investigating sleep disorder-associated functional changes in the brain. In addition, the resting-state fMRI studies of circadian rhythm disorder typically involve patients with sleep disorders or sleep deprivation. There is currently no research on brain function changes among healthy participants with purely circadian rhythm disorder.

Melatonin secretion is modulated by various factors; among them, light, which inhibits its secretion, has the strongest effect (11, 12). Using differential blood oxygenation level-dependent (BOLD) functional magnetic resonance imaging (fMRI), Vimal et al. found that the activation of the human suprachiasmatic area across times of day matched the known rhythm in the responsiveness of the circadian system to light (13). McGlashan et al. further found a positive correlation between the activation of the human suprachiasmatic area in response to light and melatonin suppression using BOLD fMRI (14). The suprachiasmatic nucleus is an anterior hypothalamic nucleus constituting the central system for regulating circadian rhythm in mammals. It projects strongly to the paraventricular nucleus (PVN), thalamus, lateral hypothalamus, dorsal medial hypothalamus, striatum, and supraventricular area. Hypothalamic nuclei, particularly the suprachiasmatic nucleus, regulates biological rhythms of the body, including body temperature, sleep/wake rhythm, hormones, metabolism, reproduction (11, 15), and melatonin secretion. Studies also have shown that the maintenance of sleep and awakening and conversion from one state to another are effectuated by a variety of neurons scattered in multiple brain regions. These neurons coordinate with and inhibit each other to dynamically regulate sleep (16–18). The hypothalamus is thought to be the control center of circadian rhythm (19). A study assessing the relationship between hypothalamic volume and sleep disorders reported significant decreases in sleep efficiency and volume of hypothalamic gray matter in patients with Huntington's disease (HD) (20). In consideration of the hypothalamic area including the suprachiasmatic area, the hypothalamus can be used as a region of interest (ROI).

FC analysis detects abnormalities in brain functional network connectivity at baseline and reflects changes in the interaction and functional coordination among multiple brain regions. However, it is unable to precisely determine sites with abnormal activity. Regional homogeneity (ReHo) analysis of resting-state fMRI reflects the resting-state functional intensity of a certain brain region. Neuroimaging technology is increasingly being harnessed to study circadian rhythms; especially, studying FC and ReHo using resting-state fMRI provides novel approaches to examine the mechanisms of circadian rhythm disorders. The combination of the two methods can better explain and reflect the state of brain function. Previous studies have used the ReHo method to analyze the brain characteristics of normal individuals and demonstrated the existence of cerebral laterality in the resting state and lateralization differences in functional tasks (21, 22). In addition, studies have reported sex-based differences in ReHo in normal individuals and changes in ReHo value of the ipsilateral motor cortex during movement, although changes in movement frequency have minimal effects on the BOLD signal (23). It is suggested that sex may influence the analysis of brain function. According to the international classification of sleep disorders (ICSD-3), circadian rhythm sleep disorders (CRSDs) can be divided into two categories based on underlying mechanisms: intrinsic CRSDs, which comprise diseases characterized by changes in endogenous oscillators (24); and extrinsic CRSDs, which involve external environment and endogenous circadian clock misalignment. The latter category includes shift-work sleep disorder (SWSD) and jet lag. Female night shift nurses in the hospital wake up at night and sleep during the day, which opposes the normal 24-h circadian rhythm; this population constitutes a typical group with CRSDs. In this

study, we evaluated the sleep habits of female night shift and female day work nurses using the Morningness-Eveningness Questionnaire (MEQ) (25). Sleep quality and mood were assessed using questionnaires, and blood levels of melatonin and cortisol were collected at five time points. Further, fMRI examinations of participants in both groups were performed. We hypothesized that circadian rhythm disorders would lead to sleep disorders, abnormal melatonin and cortisol rhythms, and corresponding sleep-related functional brain changes in night shift workers. The purpose of this study was to evaluate changes in whole-brain ReHo and hypothalamus-seeded FC associated with circadian rhythm disorders using resting-state fMRI and to elucidate the relationships of these parameters with sleep disorders in night shift nurses.

MATERIALS AND METHODS

This study was conducted at the Neurorehabilitation Department of the China Rehabilitation Research Centre between March 2019 and May 2019. All research procedures were approved by the Ethical Committee of China Rehabilitation Research Centre and were conducted in accordance with the Declaration of Helsinki (CRRC-IEC-RF-SC-005-01). Both healthy night shift and day work nurses were recruited from the population of full-time female nurses working at the facility using advertisements.

Inclusion Criteria

The inclusion criteria for day work nurses were (1) female, 18–35 years old; (2) no night shift history; (3) no sleep disorder for at least half a year before participation; (4) regular work and rest, not staying up later than 23 p.m. (UTC + 8); (5) healthy without other nervous system diseases; (6) not taking anxiety and depression drugs and sleep-regulating medications; (7) informed consent.

For night shift nurses the inclusion criteria were (1) female, 18–35 years old; (2) long term regular night shift for at least half a year, obvious day rest, and at least 6 h of night work; (3) no other nervous system disorders; (4) not taking anxiolytics, antidepressants, or sleep-regulating medications; (5) informed consent.

Exclusion Criteria

For all participants the exclusion criteria were (1) lack of informed consent; (2) taking hypnotic drugs; (3) mental disorders such as anxiety or depression; (4) failure to cooperate with the completion of the MRI examination, or intracranial abnormalities of MRI.

Participants

All participants provided written informed consent for their enrolment in the study. Participants completed general intake information forms and questionnaire surveys, allowed blood sample collection, and underwent MRI examination. None of the participants had a history of smoking or drinking, hypertension, diabetes, hyperlipidemia, sleep apnea syndrome, cardiovascular or cerebrovascular disease, fatty liver, or atrial fibrillation. None of the participants were using contraceptives. Height, weight, and body mass index (BMI) were recorded. None of the participants had traveled across time zones or had been administered hypnotics in the 6 months prior to study participation. Participants in the night shift nurses' group were all scheduled for duty for 12 h during the night, 24 h off, and then had another 12-h night shift. All participants in this group had between 1and 8 years of night shift work experience (mean, 4.22 years). For 7 days prior to laboratory admission, the 9 day-work nurses maintained their daily routines and slept for 8 h at regular times each night under dim light conditions (<10 lux) at home.

Questionnaire Survey

Participants completed a questionnaire survey. A general information questionnaire was used to obtain information on age, education, and other characteristics; evening or morning chronotype was assessed using MEQ (25), and symptoms of depression were measured using Beck Depression Inventory (BDI) (26). Furthermore, sleep quality was assessed among all participants using Pittsburgh Sleep Quality Index (PSQI) (27), and daytime somnolence was measured using Epworth Sleepiness Scale (ESS) (28).

Measurement of Body Mass Index

The participants stood upright, looked straight ahead, and breathed calmly when their height and weight were measured. BMI (kg/m^2) is calculated as a person's weight in kilograms divided by the square of their height in meters. According to the WHO standard, participants were divided into the following five groups based on their BMI: underweight, <18.5 kg/m^2; normal weight, 18.5–24.9 kg/m^2; overweight, 25.0–29.9 kg/m^2; obese, 30.0–34.9 kg/m^2; severely obese, >35 kg/m^2.

Blood Sample Collection

Participants were scheduled to enter individual quiet rooms in the neurorehabilitation department at 20:00 (UTC + 8) and remain there until 18:00 (UTC + 8) the next day. Food and water were freely available. Light intensity in the room was set at 100 lux during the time of wakefulness and at <10 lux during sleep time. The ambient temperature was maintained at 23 ± 1°C throughout the study. Peripheral venous blood was collected five times a day at 22:00, 2:00, 6:00, 10:00, and 16:00 (UTC + 8). Peripheral venous blood collection at 2:00 (UTC + 8) was performed under minimal (∼10 lux) light intensity. All individuals reported that they fell asleep without insomnia after the blood was drawn. Samples were maintained at room temperature (22–25°C) for 30 min. After centrifugation (5,000 × g for 10 min), serum was collected and cryopreserved at −80°C until subsequent use.

Detection of Serum Melatonin and Cortisol Levels

Plasma melatonin concentrations were measured using the Human Melatonin (MT) ELISA Kit (batch No: L05015328, Wuhan CUSABIO) according to the manufacturer's instructions. Serum cortisol concentrations were measured using a Human Cortisol ELISA kit (batch No: 1012019, Shanghai MiBio) according to the manufacturer's instructions. Experimenters

were blinded to groups and general participant information. The optical density (OD) was measured at 450 nm using an enzyme-labeling instrument within 10 min. A standard curve was constructed according to the concentration and OD value of the standard product. The sample concentration was then calculated according to the standard curve equation.

Neuroimaging

After blood samples were collected at 16:00 the next day (UTC + 8), fMRI was performed at 17:00 (UTC + 8). Imaging data were acquired using a Philips Achieva 3T MRI scanner with an 8-channel head coil while participants were in a conscious state. Whole-brain coverage gradient echo-planar imaging data were acquired parallel to the AC-PC line with the following parameters: 13 min 19 s, TR = 2,000 ms, TE = 30 ms, flip angle = 90°, voxels = 3 × 3 × 3 mm^3, number of slices = 50, gap = 0.6 mm, FOV = 220 × 220 mm, and matrix = 80 × 80. Standard high-resolution 3D anatomical T1-weighted scans [sagittal acquisition, repetition time (TR) = 8.2 ms, echo time (TE) = 3.2 ms, flip angle = 8°, isotropic voxel = 1 mm, number of slices = 301, matrix = 256 × 256] were collected to facilitate co-registration. All patients were scanned by the same radiologist.

Neuroimaging Preprocessing

Preprocessing and analysis of fMRI data were performed using DPABI [Data Processing & Analysis for (Resting-State) Brain Imaging] (29). The regional mean value of quantitative calculation was extracted based on an anatomical automatic labeling (AAL) map (30). Digital Imaging and Communications in Medicine (DICOM) data collected by the device were converted into an analyzable NIFTI format. After assessing the data, time slice and head movement corrections were performed. The EPI template in Montreal Neurological Institute (MNI) space was used as the reference standard. The average image obtained after head movement correction was used as the source image to estimate the registration parameters. MRIs were spatially normalized into the T1MRI template of standard MNI using a 12-parameter affinity transformation and non-linear normalization by a 7 × 8 × 7 basis function. Finally, all data were convolved with a Gaussian kernel filter of 8 × 8 × 8 mm^3 full-width half maximum (FWHM) to improve the normality of data distribution and to compensate for inexact spatial normalization. As signals related to physiological activity are concentrated in the low-frequency band, the data were filtered with a low-frequency filter of 0.01–0.1 Hz prior to statistical analysis.

Neuroimaging Analysis

As mentioned in the introduction, the hypothalamus was selected as the seed area for FC analysis. Voxel-by-voxel analysis revealed no significant difference between the day work and night shift groups; therefore, the mean seed-based FC of the hypothalamus and mean whole-brain ReHo were compared between groups with independent sample *t*-test using the AAL map as the source of ROI. Subsequently, we investigated the association of group differences of mean seed-based FC or mean whole-brain ReHo with cortisol or melatonin levels using Spearman correlation analysis. The associations of PSQI and ESS scores were also analyzed with the mean FC and mean brain ReHo using the same test. The significance threshold of brain regions with statistically significant differences was determined as $P < 0.05$.

Statistical Analysis

Clinical parameters are presented as means and standard deviations. Continuous variables are presented as medians and first and third quartiles. The PSQI, ESS, and BDI scores, which were normally distributed, were analyzed using analysis of variance (ANOVA) and single-sample K-S test. Serum melatonin and cortisol levels were compared using an independent sample *t*-test between the two groups. Pearson or Spearman correlation analysis of PSQI score, ESS score, years of shift work, serum melatonin level, and cortisol level were identified as significantly different variables. Normally and non-normally distributed data were analyzed using Pearson correlation analysis and Spearman correlation analysis, respectively. All *P*-values were two-tailed. Statistical significance was set at a value of $P < 0.05$.

RESULTS

Demographic Characteristics of the Study Population

Nine healthy female night shift nurses aged 22–33 years (mean ± standard deviation, 27.33 ± 4.243) and nine healthy female day work nurses aged 21–36 years (mean ± standard deviation, 27.22 ± 5.167) participated in this study. All participants in the night shift group had 1–8 years of night shift work experience (mean ± standard deviation, 4.222 ± 2.224). Five-night shift nurses reported poor subjective sleep, whereas similar complaints were not reported by day work nurses. No significant difference was observed in BMI between the two groups ($P = 0.276$).

Questionnaire Analysis

BDI scores did not differ significantly between the two groups. Based on the MEQ scores, 12 of the participants were classified as the intermediate type; the remaining six participants were classified as the morning type (**Table 1**). PSQI and ESS scores of night shift nurses were significantly higher than those of day work nurses, indicating poorer sleep quality and more daytime sleepiness ($P = 0.002$ and $P = 0.004$, respectively). Pearson correlation analysis revealed positive correlations of years of shift work with PSQI ($r = 0.706$, $P = 0.033$) and ESS scores ($r = 0.674$, $P = 0.046$). A positive correlation was also observed between PSQI score and BMI ($r = 0.484$, $P = 0.042$).

Blood Sample Analysis

Melatonin levels were significantly lower in night shift nurses than in day work nurses ($P = 0.006$, **Figure 1B**). A trend toward higher cortisol levels in night shift nurses than in day shift nurses was also observed, but the difference did not reach statistical significance ($P = 0.06$; **Figure 1A**). The differences in melatonin and cortisol levels at the five timepoints were further analyzed (UTC + 8). No significant between-group differences were observed in cortisol levels at any time point. Melatonin levels were lower in night shift nurses than in day work nurses at 22:00 (UTC + 8) (**Table 2**). Cortisol levels in both groups

failed to follow a circadian rhythm. No significant effect of age on melatonin secretion was observed. There was a negative association between melatonin secretion and the number of years of shift work, predominantly at 2:00 ($r = -0.787$, $P = 0.012$) and 16:00 ($r = -0.717$, $P = 0.003$). Cortisol secretion increased with age ($P < 0.05$), but no significant effect of the number of years of shift work on cortisol secretion was noted. There was no significant correlation between the secretion of melatonin and cortisol or BMI.

Functional MRI Analysis

Changes in Resting-State Whole-Brain ReHo

Mean whole-brain ReHo analysis of the two groups revealed a decrease in the activation of the bilateral cerebellar hemispheres and an increase in the activation of the bilateral superior occipital gyrus and left superior parietal gyrus in night-shift nurses (**Table 3**). Spearman's correlation analysis revealed that higher PSQI ($r = -0.529$, $P = 0.024$) and ESS scores ($r = -0.716$, $P = 0.001$) were associated with weaker activation of the right cerebellar hemisphere. Greater activation of the left superior parietal gyrus was correlated with higher PSQI ($r = 0.539$, $P = 0.021$) and ESS scores ($r = 0.582$, $P = 0.011$). Additionally, greater activation of the right superior occipital gyrus was correlated with higher PSQI ($r = 0.522$, $P = 0.026$) and ESS scores ($r = 0.661$, $P = 0.001$). Further correlation analysis revealed that a decrease in melatonin level was significantly correlated with a decrease in cerebellar hemisphere activation ($r = 0.583$, $P = 0.011$, **Figure 2A**). No correlations of ReHo brain changes with cortisol levels or BDI scores were identified.

TABLE 1 | General information and questionnaire responses.

	Night shift nurses	Day work nurses	*P*-value
N	9	9	
Age (years)	27.33 ± 4.243	27.22 ± 5.167	0.961
Body mass index (BMI)	21.767 ± 3.310	20.033 ± 3.204	0.276
MEQ	58.89 ± 12.444	53.11 ± 5.349	0.219
Morning type	4	2	0.317
Night type	0	0	
Intermediate type	5	7	
BDI	9.78 ± 9.628	9.33 ± 10.84	0.928
ESS	8.78 ± 3.456	4 ± 2.449	0.004
PSQI	8.44 ± 3.9	3.11 ± 1.900	0.002

BMI, body mass index; PSQI, Pittsburgh sleep quality index; BDI, beck depression inventory; ESS, Epworth sleepiness scale; MEQ, morningness-eveningness questionnaire.

Changes in Resting-State FC of the Hypothalamus as the Seed Region

Significant between-group differences were observed in the right middle cingulate gyrus, right putamen, and vermis (**Table 4**), suggesting that FC between the hypothalamus and right middle cingulate gyrus, right putamen, and cerebellar vermis was more enhanced in night shift nurses than in day work nurses. No correlations of FC with PSQI scores and melatonin levels were noted. FC enhancement between the hypothalamus and vermis was associated with higher ESS scores ($r = 0.501$, $P = 0.034$, **Figure 2B**). No significant correlations of FC changes with cortisol levels and BDI scores were identified.

DISCUSSION

To the best of our knowledge, this is the first study to assess night shift nurses as the research subject to confirm the presence of circadian rhythm disorder from the aspect of hormone levels, and to identify that circadian rhythm disorder can lead to changes in brain function. We examined sleep quality and daytime somnolence in night shift nurses using sleep

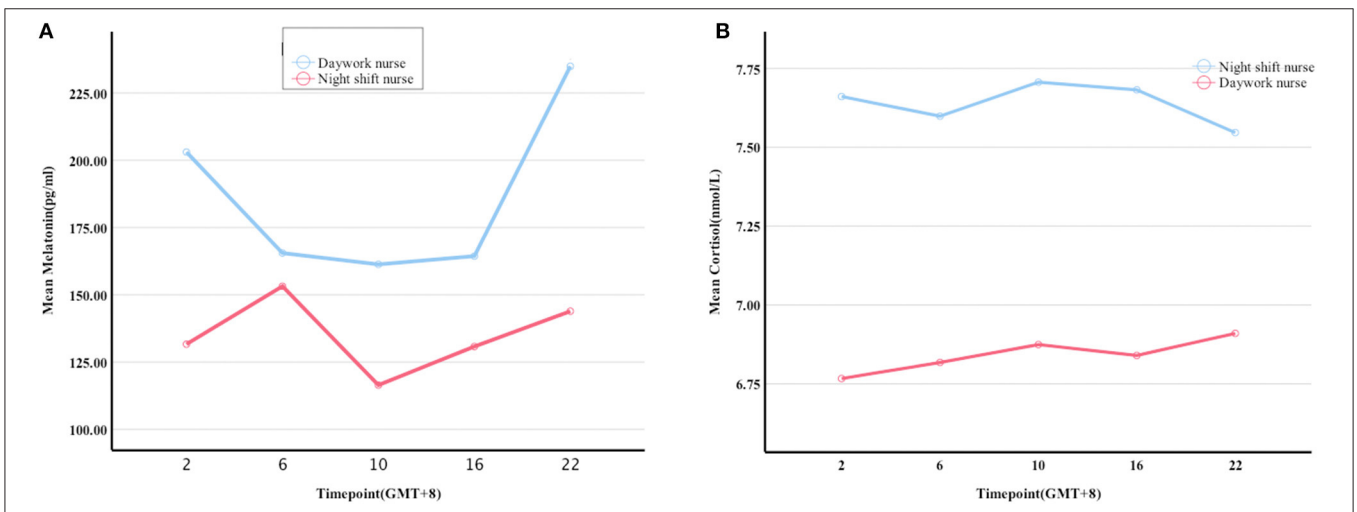

FIGURE 1 | Melatonin and cortisol levels at five timepoints within a 24 h period. **(A,B)** differences in the levels of melatonin and cortisol between the night shift and day work nurses at 2:00, 6:00, 10:00, 16:00, and 22:00 (UTC + 8), respectively.

TABLE 2 | Results of blood sample analysis.

	Night shift nurses	Day work nurses	*P*-value
N	9	9	
Cortisol (nmol/L)	7.639 ± 1.745	6.842 ± 2.203	0.060
Melatonin (pg/mL)	135.179 ± 72.504	185.832 ± 97.596	0.006
Melatonin (pg/mL) (2:00)	131.638 ± 79.141	203.048 ± 97.320	0.107
Melatonin (pg/mL) (6:00)	153.169 ± 89.659	165.514 ± 101.611	0.788
Melatonin (pg/mL) (10:00)	116.433 ± 79.587	161.308 ± 97.889	0.302
Melatonin (pg/mL) (16:00)	130.772 ± 58.586	164.359 ± 69.621	0.285
Melatonin (pg/mL) (22:00)	143.881 ± 62.955	234.930 ± 116.005	0.055

questionnaires and measurements of serum melatonin levels. Further, we analyzed changes in whole-brain ReHo and FC to the hypothalamus using resting-state fMRI. We observed that sleep quality decreased, daytime somnolence increased, and melatonin levels were reduced in night shift nurses. Further, we noted decreased activation of the bilateral cerebellar hemisphere and increased activation of the bilateral superior occipital gyrus and left superior parietal gyrus in night shift workers. Decreased activation of the right cerebellar hemisphere was associated with decreased melatonin secretion, decreased sleep quality, and increased daytime somnolence. Considering that the suprachiasmatic nucleus is located in the hypothalamus, we selected the hypothalamus as the seed area for FC analysis and observed that FC to the right middle cingulate gyrus, right putamen, and cerebellar vermis was enhanced; in particular, the enhancement of FC between the hypothalamus and cerebellar vermis was related to the increase in daytime somnolence (**Figure 3**). Despite its small sample size, this is the first study to examine changes in the brain function of otherwise healthy individuals with circadian rhythm disorders.

Our results demonstrate that night shift work resulted in sleep disorders; the longer the experience of night shift work, the poorer the sleep quality and the higher the daytime somnolence, which is consistent with previous findings (8). Meanwhile, the poorer the sleep quality, with higher the BMI. Possible mechanisms for this association include anxiety or eating at night, resting during the day, decreased exercise, or stress. In addition, because of the small sample size, no significant difference in circadian phenotype between the two groups was detected. To explore whether circadian phenotype has an impact on the brain function of participants with circadian rhythm disorder, a larger sample size is needed, and a more in-depth study on circadian rhythm disorder must be conducted according to the "owls" and "larks" types of the MEQ scale.

Epidemiological studies have demonstrated that shift work decreases melatonin secretion, induces abnormal rhythms of cortisol secretion, and disrupts inflammatory responses (3–5). In this study, night shift nurses exhibited a typical daytime reversal and served as a typical circadian rhythm population. Analyses revealed that melatonin levels were significantly decreased at night and cortisol rhythms were perturbed, consistent with previous findings. We also observed that the longer the experience of shift work, the greater the reversal of melatonin rhythm. With an increase in age, the secretion of cortisol increased. However, we did not observe an association between increased time engaged in night-shift work and cortisol levels, nor was there a significant correlation between BMI and cortisol levels, which is inconsistent with previous findings (31–34). Our failure to detect an association may be related to the small sample size.

Notably, the overall cortisol secretion rhythms of control participants in this study differed from normal rhythms, possibly due to the sample collection method. In our study, blood sample collection was conducted at five timepoints, which may have contributed to an increase in cortisol levels during collection. Melatonin or cortisol levels can be analyzed using blood, urine, hair, feces, or saliva samples. Most studies on melatonin or cortisol used urine or saliva samples (4, 5, 35), and evaluation of salivary samples is preferred to blood samples because venipuncture is associated with elevated cortisol levels (36). This may also partly explain the disruption of cortisol rhythms in day work nurses. Peripheral blood collection methods used in other studies typically involved placement of an intravenous catheter for blood sampling in the forearm vein of participants to avoid stress and to minimize impact on sleep (37). This approach should be adopted in the future to improve blood sample collection methods.

The FC of human brain networks underpins cognitive function (38, 39). Sleep and circadian rhythm disorders may interfere with cognitive processes, and perturbations of FC are associated with sleep and nervous system diseases. Facer-Childs et al. categorized early and late circadian phenotypes using the Munich Chronotype Questionnaire. Those authors selected the DMN as the seed area, which is easily affected by sleep onset and sleep deprivation. Their results indicated fundamental differences in the DMN between early and late circadian phenotypes. Resting-state FC of the DMN of late circadian phenotypes displayed dysfunction, that is decreased attention and increased subjective sleepiness (9). Indeed, sleep disorders and circadian rhythm disorders can lead to changes in brain FC. Circadian rhythm and homeostasis affect resting-state FC but are not affected by circadian typology (40). Changes in resting-state brain FC have been reported in various nervous system diseases with circadian rhythm disorders, such as delirium, Huntington's disease, and bipolar disorder (15, 20, 41). Park et al. investigated the regional cerebral blood flow in shift workers using perfusion MRI (pMRI) and observed that it decreased significantly in the cuneus, fusiform/ parahippocampal gyri, and cerebellum of the right hemisphere, while it was increased in the inferior occipital gyrus of the left hemisphere. Moreover, perfusion changes in shift workers were significantly correlated with depression and insomnia severity (8). These studies support the notion that circadian rhythm disorders affect functional brain change. McGlashan et al. reported a positive correlation between BOLD fMRI activation in the human suprachiasmatic area in response

TABLE 3 | Differences in ReHo area in the resting state between night shift and day work nurses.

Location in AAL map (19)	Brain area	Mean ± SD	Mean ± SD	*T*	*P*-value
Cerebelum_Crus1_left	Left cerebellar hemisphere	−0.0544 ± 0.1329	0.1681 ± 0.2798	−2.156	0.047
Cerebelum_Crus1_right	Right cerebellar hemisphere	−0.2372 ± 0.2506	0.1555 ± 0.2078	−3.620	0.002
Occipital_Sup_left	Left superior occipital gyrus	0.8023 ± 0.2199	0.5333 ± 0.2945	2.195	0.043
Occipital_Sup_right	Right superior occipital gyrus	0.9401 ± 0.1996	0.5784 ± 0.3896	2.480	0.025
Parietal_Sup_left	Left Superior parietal gyrus	0.4253 ± 0.1934	0.1385 ± 0.1920	3.157	0.006

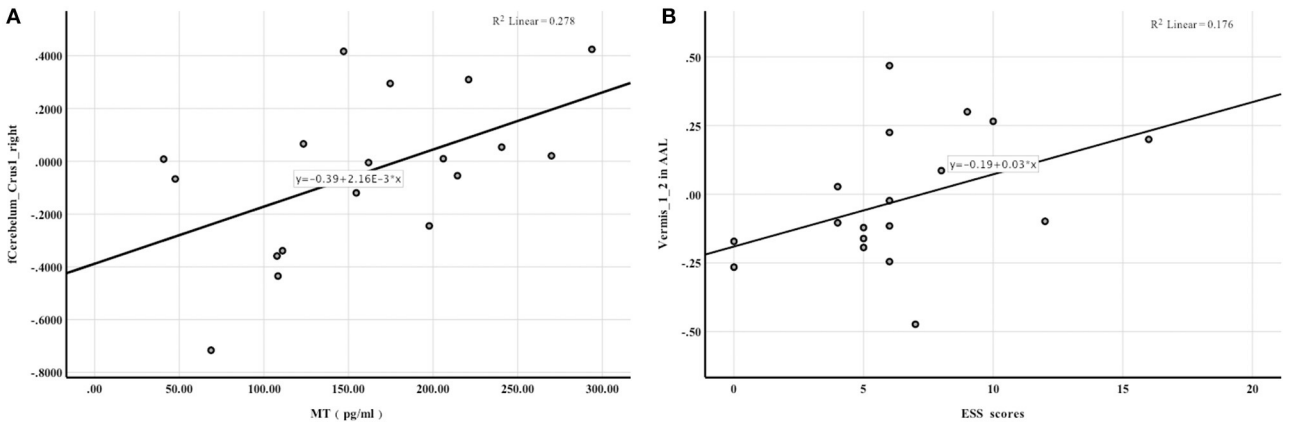

FIGURE 2 | Spearman rank correlation analysis. **(A)** the activation of Cerebelum_Crus1_right in AAL was positively correlated with melatonin levels; **(B)** FC enhancement between the hypothalamus and Vermis_1_2 in AAL was associated with higher ESS scores.

TABLE 4 | Differences in the resting-state FC area between night shift and day work nurses.

Location in AAL (19) map	Brain area	Mean ± SD	Mean ± SD	*T*	*P*-value
Cingulum_Mid_right	Median cingulate and paracingulate gyri	−0.0271 ± 0.1692	−0.1726 ± 0.1136	2.141	0.048
Putamen_right	Right putamen	0.0511 ± 0.1059	−0.0920 ± 0.1345	2.309	0.023
Vermis_1_2	Cerebellar vermis	0.0930 ± 0.2325	−0.1374 ± 0.1917	2.295	0.036
Vermis_3	Cerebellar vermis	0.0692 ± 0.1768	−0.0972 ± 0.1398	2.216	0.042

to light and melatonin suppression (9). Our results revealed a difference in mean brain ReHo between night shift and day shift nurses, and poorer sleep quality was related to weaker activation of the right cerebellum, and greater activation of the left superior parietal gyrus and right superior occipital gyrus. Moreover, the decrease in melatonin level was associated with the decrease in sleep quality as well as the decrease in the activation of the right cerebellum. The activation of the right cerebellum decreased after circadian rhythm disorder in night shift nurses, which was related to poor sleep quality, and may be involved in melatonin pathway mediated sleep regulation. Furthermore, resting-state FC also suggested that the FC between the cerebellar vermis and the hypothalamus was enhanced and this enhancement was associated with an increase in daytime sleepiness. Most studies suggest that the cerebellum may also be involved in the regulation of the sleep-wake cycle. For example, the most significant pathological change in patients with spinocerebellar ataxia is extensive degeneration of the cerebellum, and these patients often experience daytime sleepiness (42, 43). In addition, after bilateral cerebellar peduncles were damaged in cats, their awakening time was significantly reduced, and their drowsiness increased (44). Further, patients with chronic insomnia, fatal familial insomnia, obstructive sleep apnea, and daytime sleepiness exhibit a decrease in cerebellar volume (43, 45). This suggests that the cerebellum participates in the regulation of the sleep-wake cycle. Indeed, the cerebellum has been proposed to project to functional sleep areas of the thalamus and hypothalamus (46–48). Additionally, deep cerebellar nuclei form synaptic connections with multiple brain regions involved in arousal initiation and/or maintenance, such as the ventral thalamus, which are thought to promote the conversion from sleep to arousal (49, 50). Collectively, these findings suggest that activation of the cerebellum is related to sleep disorders. Combined with this, our findings may suggest that circadian rhythm disorder leads to cerebellar dysfunction and that the FC between the cerebellum and thalamus is enhanced. In our FC analysis, we did not observe any correlation

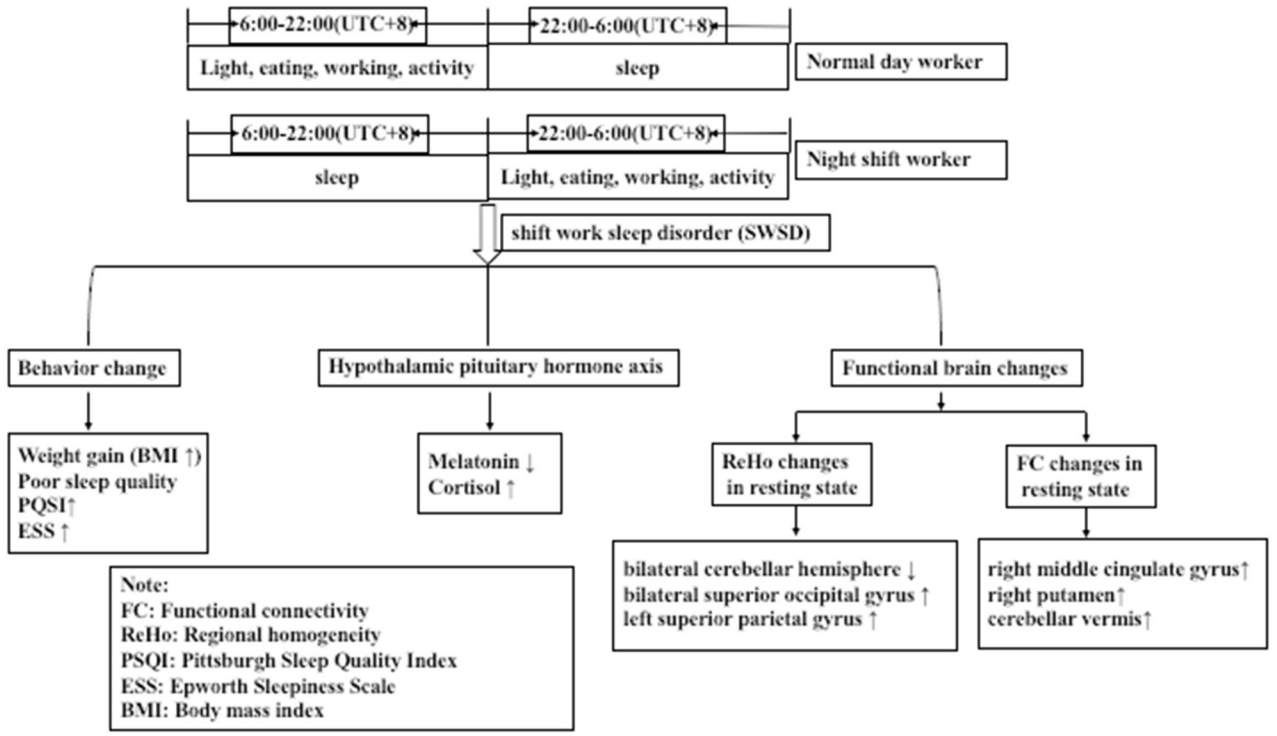

FIGURE 3 | Night shift nurses exhibited greater sleep disorders, hormonal disorders, and changes in the activation of functional brain areas than did day work nurses.

between FC change with melatonin level or PSQI score, but a positive correlation was observed between enhanced cerebellar vermis FC and ESS scores, suggesting that enhanced FC between the hypothalamus and cerebellar vermis was associated with daytime subjective sleepiness. However, it is not clear whether this suggests that the enhancement of FC between the thalamus and cerebellum is related to compensatory sleep during the daytime. In order to further elucidate this issue, a larger sample size is needed. In addition, future research can combine EEG and fMRI to detect differences in the sleep-related brain regions between participants with circadian rhythm disorders and healthy participants to determine the functional changes in these different brain areas across the sleep-wake cycle using a larger population.

In our study, night shift nurses displayed a trend of higher cortisol level and weight gain than did day shift nurses, but did not differ significantly in terms of depression; furthermore, no obvious functional brain change was observed with depression in the former group. The possible reason is that the BDI score is not sensitive to early symptoms and cannot assess anxiety and other emotional disorders. Another explanation for the inconsistency between our findings and previous research is the small sample size. Recent studies have demonstrated that sleep influences plasticity in the visual cortex. Sleep deprivation reduced task-related activity in the frontal and parietal regions as well as visuospatial attention-related and task-related activity (51). Our results indicated greater activation of ReHo in the right superior occipital gyrus and the left superior parietal gyrus. Compensatory mechanisms such as subjective effort may partially improve the level of attention and maintain certain operational ability. In this regard, compensatory efforts may have occurred in relation to the increase in ReHo in the left superior parietal gyrus and right superior occipital gyrus in the night shift nurses.

The basal forebrain is implicated in various bodily functions, including awakening, circadian rhythm, water drinking, body fluid balance, and feeding (52, 53). The putamen, a component of the basal forebrain connected to the thalamus and hypothalamus, regulates sleep and wakefulness. Decreased putamen gray matter volume, as well as a negative correlation between putamen atrophy and arousal index, has previously been reported in patients with insomnia (8). Therefore, increased connectivity between the putamen and hypothalamus may be implicated in the regulation of sleep-wake disorders. The cingulate gyrus is a component of the limbic system; the hypothalamus is connected to the cingulate gyrus via projection fibers of the papillary body and has an integrated role in the regulation of behavior, cognition, and emotion. Lesions of the cingulate gyrus result in various symptoms, such as inattention, cognitive dysfunction, and apathy. Cingulate gyrus dysfunction is noted in patients with sleep deprivation or delirium (7, 10, 54). In this study, we observed that FC between the hypothalamus and putamen, right cingulate gyrus was enhanced. Night shift nurses had obvious complaints of decreased daytime attention or increased daytime sleepiness. Although no significant difference

in BDI scores between the two groups was noted, the increased FC from the hypothalamus to the cingulate gyrus may be a compensatory mechanism to adjust emotional responses accordingly. Some studies have explored changes in brain function after 36 h of sleep deprivation and their correlation with the findings of psychomotor vigilance tests (PVT). The FC between cerebellum and central posterior gyrus decreased, which is negatively correlated with the prolongation of PVT response time; furthermore, the FC between cerebellum and bilateral caudate nucleus was enhanced, which is positively correlated with prolongation of PVT response time, suggesting that the change in cerebellum FC may be related to the impairment of psychomotor vigilance after sleep deprivation. The cerebellum is not only involved in sleep regulation, but also in cognitive functions such as responsiveness and alertness (55). Another study was conducted with 22 female patients with acute sleep deprivation. The brain activity related to sleep deprivation was analyzed based on the amplitude of low-frequency fluctuations (ALFF). The results indicated that attention decreased, response time increased, and ALFF of the right anterior cerebellar lobe decreased significantly. This further suggests that the cerebellum may participate in cognitive function and that this cognitive function might be stabilized and enhanced by sleep (56). Canto summarized that learning-related time, procedural memory formation, and spatiotemporal predictions of motor actions, which are known to be controlled at least in part by the cerebellum, are facilitated by sleep (57). Accordingly, these results led to a theoretical model of sleep-dependent memory consolidation, and the possible mechanism of memory consolidation related to the cerebellum during sleep (57). In future research, it is important to study the correlation of abnormal functional activation in brain areas associated with emotion, cognition, and sleep in a large sample of participants with circadian rhythm disorders.

Although we recruited nurses from the same work environment and same department to exclude differences in sex and levels of work stress, to the best of our ability, potential differences that may be present among our participants remain the main limitations of our study. Additional limitations include the inclusion of only female participants and small sample size. As such, the results may not be fully generalizable to males. Furthermore, significant differences in attention and emotion may be detected in a larger sample.

In conclusion, dysrhythmia of circadian rhythms contributes to resting-state functional changes in the cerebellum. In severe cases, this may be accompanied by functional changes in attention and emotion-related brain regions. In addition, for this type of population with circadian rhythm disorder, further investigation into whether transcranial magnetic stimulation can effectively regulate the abnormal activation area is warranted.

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/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethical Committee of China Rehabilitation Research Centre and were conducted in accordance with the Declaration of Helsinki (CRRC-IEC-RF-SC-005-01). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

XW was the study coordinator and contributed to the development of the study idea, analysis of demographic data and results of questionnaires and actigraphy, discussion of analysis and results, and writing and revision of the manuscript. YW contributed to the scripting of data preprocessing, as well as writing and revision of the manuscript. FB contributed to fMRI data acquisition, blood sample analysis, and revision of the manuscript. HL, YC, and LZ contributed to the collection of blood samples, analysis of demographic data and questionnaire results, and revision of the manuscript. LL contributed to the revision of the manuscript. TZ contributed to the study idea, the discussion of analysis and results, and revision of the manuscript. All authors contributed to the article and approved the submitted version.

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Characteristics of Objective Sleep and Its Related Risk Factors Among Parkinson's Disease Patients With and Without Restless Legs Syndrome

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Objective: This study aimed to investigate the objective sleep characteristics and their related risk factors among Parkinson's disease (PD) patients with and without restless legs syndrome (RLS).

Methods: A total of 125 patients with PD who underwent overnight polysomnography (PSG) were recruited consecutively. Eighty-one patients, including 27 PD with RLS (PD-RLS) and 54 PD without RLS (PD-NRLS), were included in the final analysis after 1:2 propensity score matching. Demographic, clinical, and polysomnographic data were compared between PD patients with and without RLS. The risk factors for sleep quality were examined using a multiple linear regression model.

Results: The prevalence of RLS among PD patients was 28.0% (35/125). The PD-RLS group exhibited a higher score for the Unified Parkinson Disease Rating Scale (UPDRS) III than the PD-NRLS group. Also, the PD-RLS patients displayed significantly shorter total sleep times, worse sleep quality, decreased stage 3 duration, a longer wake time after sleep onset, and a higher arousal index than those without RLS (all $p < 0.05$). In the multiple linear regression model, PD duration ($\beta = -0.363$, 95% CI: -0.652 to -0.074; $p = 0.016$), UPDRS-III ($\beta = -0.356$, 95% CI: -0.641 to -0.071; $p = 0.016$), and periodic limb movement index (PLMI) ($\beta = -0.472$, 95% CI: -0.757 to -0.187; $p = 0.002$) were determined to be the risk factors influencing sleep quality in PD-RLS patients. The UPDRS-III ($\beta = -0.347$, 95% CI: -0.590 to -0.104; $p = 0.006$) and HAMD scores ($\beta = -0.343$, 95% CI: -0.586 to -0.100; $p = 0.007$) were significantly associated with sleep quality after adjusting for confounding factors in PD-NRLS patients, respectively.

Conclusions: PD-RLS patients exhibited more disturbed and fragmented sleep in objective sleep architecture than PD-NRLS patients. The severity of motor symptoms in PD was significantly associated with poor sleep quality in both PD-RLS and PD-NRLS patients. Notably, our findings indicated that periodic limb movements during sleep (PLMS) was the risk factor that influenced the objective sleep quality in PD patients with RLS.

Keywords: Parkinson's disease, restless legs syndrome, polysomnography, periodic limb movements in sleep, objective sleep quality

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INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disease among people older than 65 years old in China (1). It is a movement disorder characterized by motor symptoms such as a resting tremor, rigidity, bradykinesia, and postural instability. Currently, clinicians have become increasingly aware of the management of non-motor symptoms of PD due to their significant impact on the quality of life of patients (2). Sleep disturbances appear to be the most frequent non-motor symptoms, as they are observed in up to 90% of PD patients (3, 4). The categories of sleep disturbances in patients with PD comprise insomnia, daytime sleepiness, restless legs syndrome (RLS), rapid eye movement sleep behavior disorder (RBD), and so on. The regulation of sleep and wakefulness is affected by the dysfunction of multiple brain areas and neurotransmitters in patients with PD (5).

RLS is a sleep disorder characterized by a series of sensorimotor symptoms and an irresistible urge to move the limbs. These symptoms are usually accompanied by uncomfortable and unpleasant sensations in the legs. Moreover, these symptoms start or worsen during periods of rest or inactivity and are relieved by movement (6). RLS is also a common sleep disturbance affecting a considerable number of PD patients. The global prevalence of RLS in PD patients was about 14–16% (7), and a more recent review indicated that the prevalence of RLS in PD patients could be as high as 52.3% (5), which was mostly higher than the prevalence of 1.9–4.6% in the general population (8). A longitudinal study reported that the prevalence of RLS increased from 4.6 to 16.3% during the progression course of PD (9). Notably, both disorders respond well to dopaminergic replacement therapy (10, 11) and share an association with periodic leg movements during sleep (12), which strongly suggest commonalities in the pathogenesis association between PD and RLS. Thus, far, it is not clear whether RLS is a manifestation of the early pathological process of PD or occurs with the progression of PD. Therefore, the hypothesis of dysfunction of the central dopaminergic system in RLS should be verified in the development of RLS in PD in the future study.

It has been reported that PD patients with RLS (PD-RLS) experience more severe sleep problems, exhibiting a significantly higher Pittsburgh Sleep Quality Index (PSQI) score and lower Parkinson's Disease Sleep Scale (PDSS) score than PD patients without RLS (PD-NRLS) (13, 14). Recently, one study indicated that PD-RLS might be related to more severe Parkinsonism, depression, cognitive dysfunction, poor sleep quality, and a worse quality of life (15–17). However, considering that the diagnosis of RLS is usually based on clinical symptoms, few studies of sleep quality in PD-RLS are included in objective assessments. In the current study, we evaluated the objective sleep quality and explored the potential influencing factors on sleep among PD patients with or without RLS.

MATERIALS AND METHODS

Study Design and Participants

This retrospective observational study was performed between January 2015 and January 2020 at the Sleep Medicine Center and the Neurology Department of the Tangdu Hospital of the Fourth Military Medical University. This study was a part of a cross-sectional study that examined sleep disorders of patients with PD. The study was conducted under the Declaration of Helsinki and was approved by the Local Ethics Committee and Institutional Review Board of the Tangdu Hospital of the Fourth Military Medical University.

In this cross-sectional study, all the hospitalized patients with PD were consecutively included and received PSG assessments regardless of any complaints of sleep disorders. A total of 125 PD patients were recruited into the study. Thirty-six patients were excluded because of incomplete clinical information or polysomnography (PSG) data. After screening, 27 PD patients were identified to exhibit RLS, and 62 PD patients did not have RLS. To reduce selection bias and potential baseline confounding factors, propensity score matching (PSM) was used to adjust the baseline clinical characteristics. Age and the levodopa equivalent dose (LED) were included in the PSM model to assess their possible influences on sleep quality. The body mass index (BMI) was used for balance calculations. PD patients with and without RLS were matched 1:2 without replacement using a nearest-neighbor approach and caliper restrictions (0.4). Finally, 27 PD patients with RLS (PD-RLS group) and 54 PD patients without RLS (PD-NRLS group) were included in the statistical analyses (**Figure 1**).

Clinical Evaluation

Demographic characteristics, including age, age at onset of PD, gender, and BMI, were collected. Disease duration and medication history were reviewed from the patients' medical records. The diagnosis of PD was based on Movement Disorder Society clinical diagnostic criteria for PD (18). The motor symptoms and the PD stage were evaluated using the Unified Parkinson Disease Rating Scale (UPDRS) III and the Modified Hoehn and Yahr Scale, respectively (19). RLS was confirmed according to the 2014 International Restless Legs Syndrome Study Group (RLSSG) diagnostic criteria: (1) the urge to move one's extremities due to uncomfortable sensations or pain; (2) the urge starts or worsens during periods of rest or inactivity; (3) the urge is worse in the evening or night; (4) and the urge is partially or totally relieved by movement (6). Notably, the above symptoms are not caused by other medical conditions such as leg cramps, positional discomfort, venous stasis, leg edema or arthritis, and so on. The RLS severity was determined using the validated International Restless Legs Syndrome Severity Scale (IRLS) (20). The LED was calculated according to the established method (21). Epworth Sleepiness Scale (ESS) was used to assess subjective daytime sleepiness and excessive daytime sleepiness (EDS), which was defined as ESS $\geq$ 10 (22). Depressive symptoms were assessed using the Hamilton Depression Rating Scale−24 (HAMD-24), and patients with a total score equal to or >20 were considered to be experiencing depression (23, 24).

Polysomnography (PSG)

All recruited patients underwent a standardized, full-night attended, digital video-PSG assessment (Philips Respironics, Murrysville, PA, USA) according to American Academy of Sleep Medicine (AASM) recommendations (25). The PSG recording

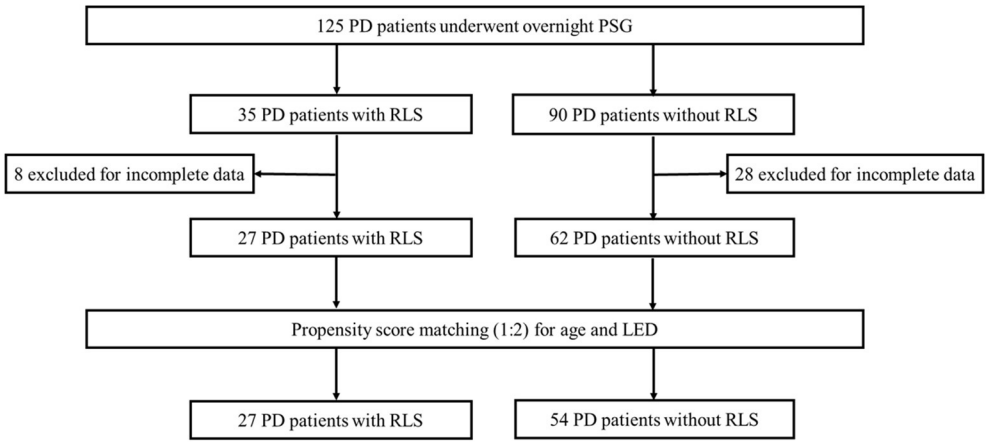

FIGURE 1 | Flow chart diagram of the selection of PD patients included in the analyses. PD, Parkinson's disease; PSG, polysomnography; RLS, restless legs syndrome; LED, levodopa equivalent dose.

included standard electroencephalogram (EEG) channels (F3-A2, F4-A1, C3-A2, C4-A1, O1-A2, O2-A1), electrooculogram (EOG), chin and bilateral anterior tibialis electromyogram (EMG), III-lead electrocardiogram (ECG), nasal-oral flow, thoracic and abdominal respiratory efforts, oxygen saturation, and body position. Sleep stages and associated events were manually scored in 30-s blocks according to the criteria described in the American Academy of Sleep Medicine (AASM) manual (25). Poor sleep quality was defined as sleep efficiency (SE) $<$ 80%. OSA was defined as an apnea–hypopnea index (AHI) $>$ 5/h with OSA-related complaints (specified in the ICSD 3rd edition) or $>$15/h without symptoms (26). Periodic limb movements during sleep (PLMS) were defined as periodic limb movement index (PLMI) $>$ 15 events per hour.

Statistical Analysis

Descriptive data were presented as means ± standard deviations or frequencies (percentages). Comparisons between two groups for continuous data were conducted using *t*-tests for normally distributed data or the Mann–Whitney *U*-test for non-normally distributed data. Categorical variables were compared using chi-square or the Fisher exact test where appropriate. Spearman's correlation analyses were used to assess the association between objective sleep parameters and potential factors that influenced sleep, including age, PD duration, UPDRS-III, HAMD, PLMI, and AHI. Multiple linear regressions were used to calculate the effects of the potentially related factors on sleep quality in the PD groups with or without RLS. All statistical analyses were conducted using SPSS version 26.0 (SPSS Inc, Chicago, IL, USA). The PSM procedure was performed using the SPSS PSM plug-in "PS Matching." A p-value < 0.05 was considered as statistically significant.

RESULTS

Demographic and Clinical Characteristics

In this study, the prevalence of RLS among PD patients was 28.0% (35/125). After screening and PSM, 81 PD patients, including 27 patients with RLS and 54 patients without RLS, were enrolled in this retrospective study. Comparisons of demographic and clinical characteristics between patients with and without RLS are presented in **Table 1**. There were no significant differences in age at the time of RLS diagnosis, sex, body mass index (BMI), age at onset of PD, and duration of PD between the groups. No differences were observed in the motor phenotype of PD, H-Y stage, LED, HAMD scores, and ESS between PD patients with or without RLS. However, patients with RLS exhibited a higher UPDRS-III score than that of patients without RLS ($p = 0.001$).

Sleep Variables

The comparisons of PSG variables between PD patients with and without RLS are shown in **Table 2**. PD-RLS patients experienced significantly shorter total sleep times, worse sleep quality, longer REM latency, decreased stage 3 duration, longer wake times after sleep onset, and a higher arousal index than those without RLS. Also, a trend toward an increased proportion of individuals exhibiting difficulty initiating sleep (SL $\geq$ 30 min) was observed in patients with RLS (40.7 vs. 22.2%, $p = 0.081$). There were no significant differences in sleep latency (SL), duration of sleep stages (S1-3 and REM sleep), AHI, and the proportion of OSA between the two groups. Interestingly, no significant differences were found in PLMI and the proportion of PLMS (PLMI > 15/h) between patients with or without RLS.

Correlations Between Sleep Parameters and Clinical Factors

Table 3 shows the correlations between sleep parameters and clinical factors in PD patients with or without RLS. The duration of PD and the UPDRS-III score were significantly negatively correlated with total sleep time (TST) and sleep efficiency in both the two groups. Interestingly, the HAMD score was negatively correlated with TST and sleep efficiency and positively correlated with time awake after sleep onset (WASO) only in PD patients without RLS. PLMI was significantly negatively correlated with TST and sleep efficiency, and positively correlated with SL in PD patients with RLS. However, there were no significant

TABLE 1 | Demographic and clinical characteristics of PD patients with and without RLS.

	All PD patients ($n = 81$)	PD-RLS ($n = 27$)	PD-NRLS ($n = 54$)	*p*
Age (years)	62.3 ± 10.0	62.4 ± 9.7	62.3 ± 10.2	0.981
Male (*n*, %)	36 (44.4)	9 (33.3)	27 (50.0)	0.235
BMI (kg/m^2)	23.3 ± 3.0	22.9 ± 3.0	23.4 ± 3.1	0.500
Age at onset of PD (years)	58.5 ± 9.9	58.1 ± 9.8	58.6 ± 10.1	0.844
Duration of disease (years)	3.7 ± 4.0	4.2 ± 4.3	3.5 ± 3.9	0.427
Motor phenotype (*n*, %)				0.717
Tremor	26 (32.1)	8 (29.6)	18 (33.3)	
Rigidity	37 (45.7)	14 (51.9)	23 (42.6)	
Mixed	18 (22.2)	5 (18.5)	13 (24.1)	
Hoehn and Yahr stage	2.3 ± 0.7	2.4 ± 0.7	2.3 ± 0.7	0.779
UPDRS-III (M)	15.6	18.3	15.6	**0.001**
LED	317.2 ± 180.0	320.8 ± 184.1	315.4 ± 179.6	0.899
HAMD	14.1 ± 5.5	14.7 ± 6.8	13.8 ± 4.7	0.468
Depression (HAMD ≥ 20) (*n*, %)	9 (11.1)	4 (14.8)	5 (9.3)	0.472
ESS	6.0 ± 5.2	6.5 ± 5.1	5.9 ± 5.3	0.598
EDS (ESS ≥ 10) (*n*, %)	25 (30.9)	10 (37.0)	15 (27.8)	0.395
IRLS	—	20.3 ± 6.4	—	—

PD, Parkinson's disease; RLS, restless legs syndrome; NRLS, without restless legs syndrome; BMI, body mass index; LED, levodopa equivalent dose; UPDRS-III, Unified Parkinson's Disease Rating Scale, Part III; M, the median; HAMD, Hamilton Depression Rating Scale; ESS, Epworth Sleepiness Scale; EDS, excessive daytime sleepiness; IRLS, International Restless Legs Syndrome Severity Scale. A p-value in bold denotes a significant difference ($p < 0.05$).

TABLE 2 | Sleep parameters of PD patients with and without RLS.

	PD-RLS ($n = 27$)	PD-NRLS ($n = 54$)	*p*
Total sleep time (min)	281.8 ± 89.0	327.7 ± 73.8	**0.016**
Sleep efficiency (%)	60.2 ± 19.1	77.5 ± 59.5	0.145
Poor sleep quality (SE < 0.8) (*n*, %)	17 (47.2)	39 (24.5)	**0.045**
Sleep latency (min)	34.9 ± 48.3	24.7 ± 23.0	0.199
Difficulty initiating sleep (SL ≥ 30 min) (*n*, %)	11 (40.7)	12 (22.2)	0.081
REM latency (min)	219.5 ± 114.4	161.1 ± 98.2	**0.027**
Stage 1 (min)	90.4 ± 41.6	94.5 ± 61.5	0.757
Stage 2 (min)	140.0 ± 65.4	159.2 ± 80.7	0.288
Stage 3 (min)	14.1 ± 16.7	27.3 ± 36.2	**0.027**
Stage REM (min)	37.3 ± 23.0	46.6 ± 35.8	0.160
WASO (min)	139.6 ± 77.5	97.5 ± 68.9	**0.015**
Arousal index (/h)	16.9 ± 12.7	9.2 ± 10.4	**0.005**
PLMI (/h)	19.4 ± 28.5	18.8 ± 30.1	0.932
PLMS (PLMI>15/h) (*n*, %)	10 (37.0)	17 (31.5)	0.617
AHI (/h)	8.9 ± 12.5	8.6 ± 12.6	0.899
OSA (n, %)	13 (48.1)	20 (37.0)	0.337

PD, Parkinson's disease; RLS, restless legs syndrome; NRLS, without restless legs syndrome; SE, sleep efficiency; SL, sleep latency; REM, rapid eye movement period; WASO, wake after sleep onset; PLMI, periodic limb movement index; PLMS, periodic limb movements during sleep; AHI, apnea–hypopnea index; OSA, obstructive sleep apnea. A p-value in bold denotes a significant difference ($p < 0.05$).

correlations between AHI and sleep parameters including TST, SE, WASO, and SL for both groups.

Risk Factors of Sleep Quality

Based on the differences observed in the correlation analysis between the two groups, multiple linear regression was conducted to explore the potential risk factors influencing objective sleep quality in PD patients with or without RLS. Sleep efficiency was considered to be the dependent variable, and various potential factors, including PD duration, UPDRS-III, HAMD, PLMI, and AHI, were independent variables (4, 27, 28). Age and gender were adjusted in the final model.

TABLE 3 | Correlations between sleep parameters and clinical factors.

	Total sleep time (min)		Sleep efficiency (%)		WASO (min)		Sleep latency (min)	
	PD-RLS	**PD-NRLS**	**PD-RLS**	**PD-NRLS**	**PD-RLS**	**PD-NRLS**	**PD-RLS**	**PD-NRLS**
Age	−0.35 (0.07)	**−0.33 (0.02)**	**−0.38 (0.05)**	**0.34 (<0.01)**	0.35 (0.07)	**0.27 (0.05)**	0.29 (0.14)	0.26 (0.06)
PD duration	**−0.50 (<0.01)**	**−0.27 (0.05)**	**−0.50 (0.01)**	**−0.28 (0.04)**	0.37 (0.06)	0.19 (0.17)	0.33 (0.09)	0.01 (0.94)
UPDRS-III	**−0.40 (0.04)**	**−0.37 (<0.01)**	**−0.41 (0.03)**	**−0.38 (<0.01)**	0.21 (0.29)	**0.34 (<0.01)**	0.16 (0.44)	0.15 (0.28)
HAMD	−0.19 (0.35)	**−0.34 (< 0.01)**	−0.14 (0.49)	**−0.38 (< 0.01)**	−0.06 (0.76)	**0.34 (<0.01)**	−0.14 (0.48)	0.11 (0.44)
PLMI	**−0.50 (<0.01)**	−0.26 (0.06)	**−0.53 (<0.01)**	−0.22 (0.11)	0.26 (0.20)	0.15 (0.30)	**0.69 (<0.01)**	0.17 (0.23)
AHI	0.26 (0.19)	−0.26 (0.06)	0.24 (0.23)	−0.26 (0.06)	−0.07 (0.74)	0.15 (0.28)	−0.23 (0.25)	0.16 (0.25)

WASO, wake after sleep onset; PD, Parkinson's disease; RLS, restless legs syndrome; NRLS, without restless legs syndrome; UPDRS-III, Unified Parkinson's Disease Rating Scale, Part III; HAMD, Hamilton Depression Rating Scale; PLMI, periodic limb movement index; AHI, apnea–hypopnea index. A p-value in bold denotes a significant difference ($p < 0.05$).

The model that considered all independent variables explained 76.9% of the variability observed in the sleep efficiency of PD-RLS patients ($R^2 = 0.701$, $p = 0.001$). When the independent variables were introduced using a stepwise method, PD duration ($\beta = -0.363$, 95% CI: −0.652 to −0.074; $p = 0.016$), UPDRS-III ($\beta = -0.356$, 95% CI: −0.641 to −0.071; $p = 0.016$), and PLMI ($\beta = -0.472$, 95% CI: −0.757 to −0.187; $p = 0.002$) were the risk factors determined to be associated with sleep efficiency in PD patients with RLS after adjusting for potential confounding variables including age and gender (**Figure 2**). In PD patients without RLS, the UPDRS-III and HAMD scores were associated significantly with sleep efficiency after adjusting for confounding factors ($\beta = -0.347$, 95% CI: −0.590 to −0.104; $p = 0.006$; $\beta = -0.343$, 95% CI: −0.586 to −0.100; $p = 0.007$, respectively) (**Figure 3**).

DISCUSSION

To the best of our knowledge, this is the first study to evaluate objective sleep characteristics and potential risk factors of PD patients with and without RLS. After matching the propensity scores of the factors (age and LED) that might have affected sleep quality in PD patients (27, 29), we observed that PD-RLS patients experienced worse nocturnal sleep quality as indicated by a shorter total sleep time, a shorter stage 3 duration, a longer WASO, and a higher arousal index than PD patients without RLS. These results were similar to previous studies that reported worse subjective sleep quality in PD patients with RLS than those without RLS (13, 14). In the current study, we identified several risk factors that influenced sleep quality, including PD duration, UPDRS-III, and PLMI in PD-RLS patients, as well as UPDRS-III and HAMD scores in PD-NRLS patients.

Sleep disorders are complex and diverse in PD patients (30) and are caused by both motor and non-motor symptoms associated with PD. It is well-known that the motor symptoms of PD *per se*, such as difficulty turning over in bed when in the unpredictable "off" state, can result in sleep disturbance associated with nocturnal awakening (31). Unsurprisingly, as a widely used tool to assess the motor performance of PD patients, UPDRS-III was found to be a significant risk factor for sleep quality both in PD-RLS and PD-NRLS patients. Therefore, our findings provided reliable evidence that treatment of sleep disturbance could include improving motor symptoms in PD patients with or without RLS. The UPDRS- III score in PD-RLS patients was significantly higher than that of PD-NRLS patients. Several studies have reported that the diencephalon-spinal pathway, especially abnormalities in the A11 dopaminergic neurons, could lead to RLS (32). Thus, PD-RLS patients might experience a wider range of neurodegeneration and show more severe motor symptoms than PD-NRLS patients.

Depression is one of the most common non-motor symptoms observed in patients with PD and has been found to be associated with worse nighttime sleep in PD (33). In our study, the results revealed that a higher HAMD score was associated with worse objective sleep quality in the PD-NRLS group but not in the PD-RLS group. Similarly, in previous studies, a higher HAMD score was significantly correlated with worse subjective sleep quality of patients with PD as assessed using the PDSS (27, 34).

This current study found that RLS was a common comorbid condition (28.0%) in PD when the International Restless Legs Syndrome Study Group (RLSSG) diagnostic criteria (6) were used. RLS has been reported to be frequent in patients with PD, but whether the prevalence of RLS in PD is higher than in the general population is still a matter of debate (5). The dopaminergic dysfunction is a potential explanation for the high prevalence of RLS in PD. As mentioned, somatic motor neuron activity is known to be mediated through the diencephalon-spinal dopamine pathway, which is proposed to play an important role in the pathophysiology of RLS. A11 neurons might degenerate in patients with PD, and the degeneration of this pathway is believed to be strongly related to the occurrence of RLS in PD patients (35). As is known, patients with RLS primarily report that their sleep disruption is caused by unpleasant sensations and usually report difficulty initiating and maintaining sleep, as well as experiencing unrefreshing sleep two to three times more often than healthy control subjects (36). Also, the objective sleep architecture as assessed by PSG presents with a longer sleep latency and a higher arousal index in RLS. It has been speculated that RLS might share underlying commonalities in pathophysiology with PD (37). Thus, it is noteworthy that differences in motor and non-motor symptoms between PD patients with and without RLS were observed. Currently, some studies have reported that PD patients with RLS exhibit more severe Parkinsonism, worse mental health, and worse sleep

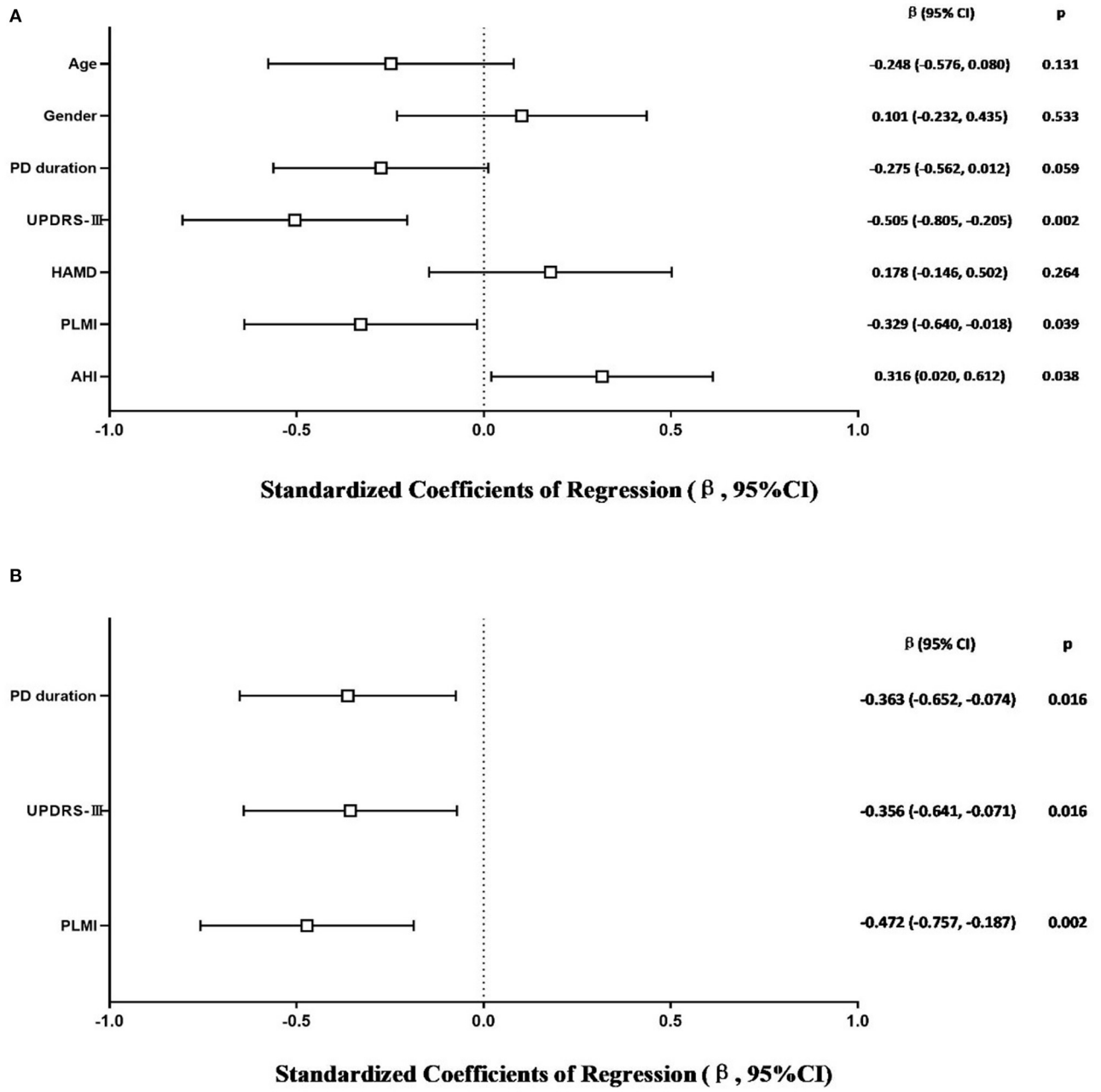

FIGURE 2 | Multiple linear regression analysis of sleep efficiency in PD-RLS patients. The squares are the standardized regression coefficients (β, the change in terms of SDs in the dependent variable that results from a change of 1 SD in an independent variable), and the error bars indicate the 95% CI of β. The gender variant was coded as a dummy variable: male was coded 0, and 1 was used for female. PD, Parkinson's disease; RLS, restless legs syndrome; UPDRS-III, Unified Parkinson Disease Rating Scale Part III; HAMD, Hamilton Depression Rating Scale; PLMI, periodic limb movement index; AHI, apnea–hypopnea index. **(A)** Risk factors of sleep efficiency in PD-RLS patients. All-factors regression ($R^2 = 0.701$, $p = 0.001$). **(B)** Stepwise regression (Age- and gender-adjusted, $R^2 = 0.577$, $p < 0.001$).

quality, using relevant assessment scales (38–40). However, no specific studies have been performed to evaluate sleep in PD patients with and without RLS using objective sleep parameters.

Therefore, this retrospective analysis was conducted on PD patients to evaluate objective sleep architecture and its differences between patients with or without RLS. Concomitant with the subjective studies, the current study observed that PD patients with RLS had worse nocturnal sleep quality compared with PD patients without RLS. In particular, our results demonstrated that PLMI was the risk factor that influenced sleep quality in the PD-RLS group after controlling for potential confounders. Thus, this study provided objective evidence for the effect of RLS on sleep quality in PD patients. Periodic limb movements (PLMs) occur in up to 80% of patients with RLS (41),

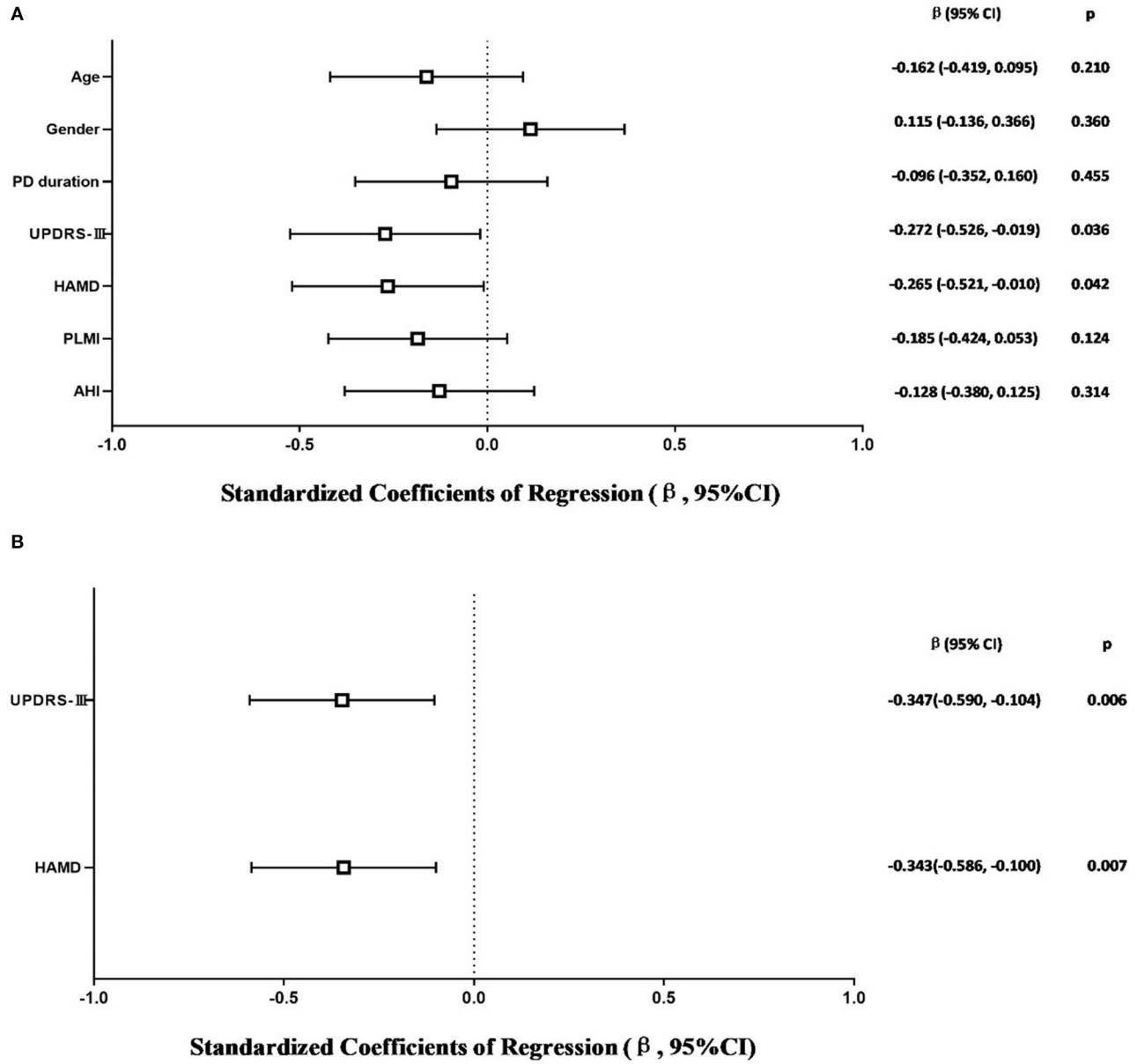

FIGURE 3 | Multiple linear regression analysis of sleep efficiency in PD-NRLS patients. The squares are the standardized regression coefficients (β, the change in terms of SDs in the dependent variable that results from a change of 1 SD in an independent variable), and the error bars indicate the 95% CI of β. The gender variant was coded as a dummy variable: male was coded 0, and 1 was used for female. PD, Parkinson's disease; NRLS, without restless legs syndrome; UPDRS-III, Unified Parkinson Disease Rating Scale Part III; HAMD, Hamilton Depression Rating Scale; PLMI, periodic limb movement index; AHI, apnea–hypopnea index. **(A)** Risk factors of sleep efficiency in PD-NRLS patients. All-factors regression ($R^2 = 0.365$, $p = 0.003$). **(B)** Stepwise regression (Age- and gender-adjusted, $R^2 = 0.260$, $p < 0.001$).

which can disrupt sleep quantity and quality (42, 43). PLMI is the indicator of PLMS, and our present results suggested that RLS adversely impacted sleep quality in PD patients when PLMI was used as an objective indicator. It has been reported that there is a high prevalence (39–57.8%) of PLMS in PD patients and that reduced striatal dopamine transporter binding and nigrostriatal dopaminergic cell loss might be the underlying reason for the comorbidity of PD and PLMS (44–46). Similarly, in our results, it could explain why there was no significant difference for PLMI between PD-RLS and PD-NRLS patients due to the common pathological mechanism. Notably, in the current study, there was a relatively low occurrence of PLMS in PD-RLS (37%) and PD-NRLS (31.5%) patients, respectively. We speculated that the dopaminergic therapy before PSG assessment in most PD patients could be the important impact factor.

The strengths of the present study include two major points. The first is the objective assessment of sleep quality using PSG.

The second is the adjustment of possible factors that influenced sleep quality at the baseline using the PSM model. However, several limitations should be noted in this retrospective study. First, a relatively small sample size could affect the power of the statistical analysis. There were only 27 patients in the PD-RLS group due to the relatively low prevalence of RLS in the study population. Second, the sleep assessment only used PSG, which might result in an overestimation of the sleep quality due to the absence of a subjective sleep evaluation. Third, this study lacked a healthy group to serve as the control. Fourth, we could not evaluate the coexistence of RBD and RLS in patients with PD due to the incomplete data in the diagnosis of RBD. Finally, the sleep architecture could be influenced in part by a "first night effect" due to the collection of data using only a single night data for PSG monitoring. Future studies that include age–sex matched healthy controls are needed to confirm the differences in sleep quality between PD-RLS and PD-NRLS patients and controls.

CONCLUSION

This study provided objective evidence that PD-RLS patients have worse nocturnal sleep than PD-NRLS patients. We also observed that several risk factors were associated with the sleep quality in PD patients with or without RLS patients. The finding that PLMS was the risk factor that influenced sleep quality in PD-RLS but not in PD-NRLS patients might suggest that PLMI could be a robust indicator of sleep disruption in PD patients with RLS.

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 Tangdu Ethics Committee and Institutional Review Board. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

SS implemented the research and collated and analyzed the data. JZ and CS supervised the research. JR responsible for data entry. XZ and JC were responsible for the quality control. All authors read and approved the manuscript.

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