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RESEARCH ARTICLE

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Altered thalamic connectivity in insomnia disorder during wakefulness and sleep

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Abstract

Insomnia disorder is the most common sleep disorder and has drawn increasing attention. Many studies have shown that hyperarousal plays a key role in the pathophysiology of insomnia disorder. However, the specific brain mechanisms underlying insomnia disorder remain unclear. To elucidate the neuropathophysiology of insomnia disorder, we investigated the brain functional networks of patients with insomnia disorder and healthy controls across the sleep–wake cycle. EEG-fMRI data from 33 patients with insomnia disorder and 31 well-matched healthy controls during wakefulness and nonrapid eye movement sleep, including N1, N2 and N3 stages, were analyzed. A medial and anterior thalamic region was selected as the seed considering its role in sleep–wake regulation. The functional connectivity between the thalamic seed and voxels across the brain was calculated. ANOVA with factors "group" and "stage" was performed on thalamus-based functional connectivity. Correlations between the misperception index and altered functional connectivity were explored. A group-by-stage interaction was observed at widespread cortical regions. Regarding the main effect of group, patients with insomnia disorder demonstrated decreased thalamic connectivity with the left amygdala, parahippocampal gyrus, putamen, pallidum and hippocampus across wakefulness and all three nonrapid eye movement sleep stages. The thalamic connectivity in the subcortical cluster and the right

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temporal cluster in N1 was significantly correlated with the misperception index. This study demonstrated the brain functional basis in insomnia disorder and illustrated its relationship with sleep misperception, shedding new light on the brain mechanisms of insomnia disorder and indicating potential therapeutic targets for its treatment.

KEYWORDS

EEG-fMRI, functional connectivity, hyperarousal, insomnia disorder, sleep misperception, thalamus

1 | INTRODUCTION

Insomnia disorder is the most common sleep disorder and affects approximately 10% of the adult population (Chung et al., 2015; Ohayon, 2002). However, little is known about the specific neural mechanisms of insomnia disorder. There has been increasing attention drawn to the hyperarousal hypothesis of insomnia disorder that patients with insomnia disorder have higher arousal levels expressed in terms of somatic, cognitive and cortical activity. It is now known that individuals with insomnia have increased heart rate, hormone levels, metabolism, high-frequency electroencephalography (EEG) power, and sympathetic system activity (Bonnet & Arand, 2010). A positron emission tomography (PET) study found that, compared with healthy controls, patients with insomnia disorder showed increased global cerebral glucose metabolism during sleep and wakefulness, as well as a smaller decline in relative metabolism from wakefulness to sleep in wake-promoting regions including the thalamus (Nofzinger et al., 2004). This PET study provided the first direct neuroimaging evidence for the hyperarousal hypothesis. A subsequent study showed a positive correlation between increased metabolism during sleep and wake time after sleep onset (WASO) in individuals with insomnia (Nofzinger et al., 2006). However, it should be noted that a later study with a larger sample size did not find differences in wake-promoting regions in patients with insomnia disorder (Kay et al., 2016). Experiments with rats suggested that stress-induced insomnia was driven by the residual activity of the arousal system instead of changes in the sleep system that remained fully active (Cano, Mochizuki, & Saper, 2008). Abnormal activity in arousal and limbic systems was found in rats with stress-induced insomnia. Moreover, lesions in these regions blocked inappropriate arousal (Cano et al., 2008; Riemann et al., 2010).

Cortical hyperarousal results have been demonstrated in abnormal sensory and information processing and unusual long-term memory formation, and they are related to sleep disturbances and sleep misperception in insomnia disorder (Riemann et al., 2010). Sleep misperception is a concept used to describe the discrepancy between subjective sleep experience measured by sleep questionnaires and objective parameters measured by polysomnography (PSG) (Feige et al., 2013). As a ubiquitous symptom of insomnia disorder (Harvey & Tang, 2012), sleep misperception has been found to be correlated with the number of cyclic alternating patterns and higher frequency activities in EEG during nonrapid eye movement (NREM) sleep (Krystal, Edinger, Wohlgemuth, & Marsh, 2002; Lecci et al., 2020; Parrino, Milioli, De Paolis, Grassi, & Terzano, 2009). It has been suggested that sleep misperception may be due to enhanced information processing related to hyperarousal (Riemann et al., 2010).

Recently, further functional magnetic resonance imaging (fMRI) studies investigated the brain mechanisms of insomnia disorder during tasks or resting wakefulness, but those studies did not reveal consistent brain alterations (Spiegelhalder et al., 2015; Tahmasian et al., 2018). These divergent results may be attributed to the lack of fMRI data during sleep; additionally, the wakeful state may include a variety of physiological states (Tagliazucchi & Laufs, 2014). A previous study demonstrated the influence of sleep onset on the thalamic functional connectivity (Hale et al., 2016) in healthy controls. A following study identified modification of thalamic functional connectivity during wakefulness and light sleep in epilepsy (Bagshaw et al., 2017). Motivated by these findings, we proposed that investigations of the brain thalamic networks across wakefulness and different sleep stages in patients with insomnia disorder will help to elucidate the neural circuitry underlying insomnia disorder.

The thalamus is a major part of the ascending reticular activating system (Moruzzi & Magoun, 1949). Thalamic activity has been closely related to the transition from NREM sleep to wakefulness, arousals during NREM sleep, recovery from anesthesia, and vigilance level (Balkin et al., 2002; Falahpour, Chang, Wong, & Liu, 2018; Langsjo et al., 2012; Zou et al., 2020). Severe atrophy of the medial dorsal and anterior thalamus was reported in patients with familiar fatal insomnia (Cracco, Appleby, & Gambetti, 2018; Lugaresi et al., 1986). A lesion study in cats provided evidence for the role of the medial dorsal thalamus in sleep–wake regulation (Marini, Imeri, & Mancia, 1988). Some patients with paramedian thalamic syndromes showed dysfunction in sleep–wake regulation (Montagna, Gambetti, Cortelli, & Lugaresi, 2003). The medial dorsal and anterior nuclei of the thalamus are major parts of the limbic thalamus, which is involved in memory, emotion, and arousal (Taber, Wen, Khan, & Hurley, 2004). Thus, the medial and anterior thalamic nuclei are functionally closely related to the arousal system and limbic system, both of which showed abnormal activity in rats with insomnia (Cano et al., 2008; Riemann et al., 2010). A thalamic seed that was derived from functional meta-analysis and located around the medial and anterior thalamus was adopted to perform functional connectivity analysis. We hypothesize that this thalamic

region has altered connectivity with the arousal and limbic systems in patients with insomnia disorder, which serves as the brain network basis for cortical hyperarousal and interprets certain clinical sleep parameters of insomnia disorder.

2 | MATERIAL AND METHODS

2.1 | Participants

Participants in this study were recruited from the right-handed Chinese Han population between July 2017 and September 2019. Patients with insomnia disorder were diagnosed using a structured clinical interview according to the Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-5), criteria (American-Psychiatric-Association, 2013). All the patients recruited had a duration of insomnia for longer than 3 months (i.e., persistent insomnia), and they were experiencing symptoms of insomnia at the time of participation in this study. The matched healthy controls had no insomnia symptoms and met the criteria for normal sleep (Beattie, Espie, Kyle, & Biello, 2015). These groups were matched for age, sex, and years of education. Participants were asked to complete questionnaires, including PSQI (Pittsburgh sleep quality index), SDS (Self-Rating Depression Scale), and SAS (Self-Rating Anxiety Scale) during the recruitment process.

The exclusion criteria were as follows: (a) age under 18 years old or over 60 years old, (b) education years <7, (c) personal or family history of psychiatric or neurological illness (other than insomnia disorder for the insomnia disorder group), (d) current or previous drug or alcohol use disorder, (e) PLMA (periodic limb movements with awakening) ≥ 20/hr, (f) AHI (apnea-hypopnea index) ≥ 5, (g) MMSE (mini-mental state examination) score < 27, (h) use of drugs that influence sleep within the previous 2 weeks, (i) body mass index ≥35, and (j) shift work. This study was approved by the Institutional Review Board of Peking University Sixth Hospital. Written informed consent was obtained from each participant.

2.2 | Experimental design

Participants were asked to follow a regular sleep schedule (at least a regular on-bed time for patients with insomnia disorder) for 2 weeks, and their habitual sleep patterns were monitored through actigraphy and sleep diary. Subsequently, PSG (Grael, Compumedics, Australia) data for two consecutive nights were collected at Peking University Sixth Hospital to evaluate sleep structure and to exclude participants with other sleep disorders (two participants in the insomnia group and one participant in the control group had only one night of PSG data). To promote sleep and to reduce the first-night effect, an adaptation session to our MR scanner (3T Prisma Scanner, Siemens Healthineers, Erlangen, Germany) was conducted at the Center for MRI Research, Peking University. Participants were instructed to lie in the scanner wearing a 64-channel MR-compatible EEG cap (Brain Products, Munich, Germany) and underwent 6 min of T_1-weighted scanning and 30 min of BOLD fMRI scanning. Within a week after the adaptation session, the experimental sleep recordings using simultaneous EEG-fMRI were conducted at the participants' usual bedtime (ranging from 8:30 p.m. to 1:00 a.m.). Notably, no sleep deprivation was involved.

2.3 | EEG-fMRI data acquisition

EEG-fMRI data for wakefulness and NREM sleep during the night were collected from 33 patients with insomnia disorder and 32 age- and sex-matched healthy controls using the 3-Tesla Prisma Scanner and the 64-channel MR-compatible EEG system. The subjects were asked to lie supine in the scanner during the entire scanning session. Earplugs and cushions were provided for noise protection and head motion restriction. The resistance of the reference and ground channels was kept below 10 kΩ, and the resistance of the other channels was kept below 20 kΩ. The recording sampling rate was 5,000 Hz, and the data were filtered with a low cutoff of 10 s and a high cutoff of 250 Hz. The resistance of all the channels was verified again before the start of MR scanning. Wires connecting the cap and the amplifiers were fixed to avoid any potential vibration during the MR scan.

For registration purposes, high-resolution T_1-weighted anatomical images were acquired using a three-dimensional magnetization-prepared rapid acquisition gradient-echo sequence (repetition time (TR) = 2,530 ms, echo time (TE) = 2.98 ms, inversion time = 1,100 ms, flip angle (FA) = 7°, number of slices = 192, and voxel resolution = $0.5 \times 0.5 \times 1\ mm^3$). The participants were asked to lie quietly in the scanner during data acquisition.

Then, the "sleep" session began after the participants were instructed to try and fall asleep. The sleep BOLD fMRI data sets were collected using a gradient echo-planar imaging sequence (TR = 2000 ms, TE = 30 ms, FA = 90°, number of slices = 33, slice thickness = 3.5 mm, gap = 0.7 mm, matrix = 64 × 64, and in-plane resolution = $3.5 \times 3.5\ mm^2$). The "sleep" session ended when the participants were completely awake and could not fall asleep again or until all 4,096 volumes (the largest number of volumes that could be acquired in a run for the BOLD fMRI sequence in our scanner, that is, 8,192 s because 2 s was required to acquire one volume in this session) had been acquired. Considering the relatively long scan time, the temporal signal-to-noise ratio was checked and was stable across the scan (Supporting Information Methods, Figure S1). The EEG and fMRI data were synchronized in terms of triggers (SyncBox, Brain Products).

2.4 | EEG data preprocessing and sleep stage scoring

EEG data preprocessing was performed using BrainVision Analyzer 2.1 (Brain Products). MR gradient and ballistocardiogram artifacts in the EEG data were removed by the average artifact subtraction method (Allen, Josephs, & Turner, 2000). Data were downsampled to 500 Hz while MR gradient removal was performed. Then, the data

were rereferenced to the mean of the channels at the mastoids and temporally filtered (10–100 Hz for electromyogram channels and 0.3–35 Hz for the other channels). Sleep stages were scored by two experienced technicians based on the American Academy of Sleep Medicine criteria (Iber, Ancoli-Israel, Chesson, & Quan, 2007). Only 30-s epochs with scores consistent between the scorers were used for further analysis.

2.5 | FMRI data processing

FMRI data of continuous 5-min epochs of stage wakefulness, N1, N2, and N3 were extracted. It was shown that using 5-min data was sufficient to estimate stable correlation strengths (Van Dijk et al., 2010). However, it should be noted that a later study suggested that increased data length can further improve reliability (Birn et al., 2013). However, there was a tradeoff between epoch length and number of epochs. In this study, a longer epoch would result in fewer epochs available. In addition, the criterion of a 5-min epoch was used in several previous studies in sleep (Hsiao et al., 2018; Mitra et al., 2016; Samann et al., 2011; Spoormaker et al., 2010; Tagliazucchi et al., 2013). Thus, we used data of 5-min epochs to perform the following analysis.

The fMRI data were preprocessed using the FMRIB Software Library (FSL5.0.9 https://fsl.fmrib.ox.ac.uk/fsl/fslwiki) (Jenkinson, Beckmann, Behrens, Woolrich, & Smith, 2012) and the CONN toolbox (CONN18.b https://www.nitrc.org/projects/conn/) (Whitfield-Gabrieli & Nieto-Castanon, 2012). The preprocessing steps included slice timing, motion correction, spatial normalization (voxel size, $2 \times 2 \times 2$ mm^3) to the International Consortium for Brain Mapping standard template, smoothing (full-width at half-maximum, 6 mm), regressing nuisance signals including the Friston 24 motion parameters (six head motion parameters and their first derivative, and the corresponding squared items) (Friston, Williams, Howard, Frackowiak, & Turner, 1996) and six primary components of cerebral spinal fluid derived using the CompCor method (Behzadi, Restom, Liau, & Liu, 2007), linear detrending and temporal filtering (0.008–0.09 Hz).

The thalamic seed was selected based on the Power264 template (Power et al., 2011) (Figure 1). We selected this region as seed based on the fact that this template was derived from a functional meta-analysis and the selected region of interest (ROI) was located bilaterally around the anterior and medial thalamus. In contrast, several other thalamus atlases are mainly based on functional or structural connectivity between thalamic voxels and several major cortices (Behrens et al., 2003; Hale et al., 2015; Ji et al., 2016; Johansen-Berg et al., 2005). Correlation coefficients between each voxel across the brain and the thalamic seed were calculated, and Fisher's z-transform was applied. We overlapped the seed with a thalamic atlas derived from histological data (Jakab, Blanc, Berenyi, & Szekely, 2012; Krauth et al., 2010) and showed that the seed mainly included anterior ventral, medial dorsal, anterior dorsal and lateral dorsal nuclei (Table S1). It should be noted that the seed seems to extend into the cerebrospinal fluid (CSF) and many voxels were not included in the atlas. Therefore, we then checked the seed with tissue probability maps in Statistical Parametric Mapping (SPM12, http://www.fil.ion.ucl.ac.uk/spm/). Among the 311 voxels in the seed (2 mm resolution), only six voxels had a probability larger than 0.5 for CSF, and 243 voxels had the greatest probability as gray matter. We performed additional analysis using the voxels in gray matter for the seed shown in Figure 1 and the thalamic seed derived using independent component analysis (Supporting Information, Figures S2 and S3).

2.6 | Statistical analysis

The program 3dLMEr (https://afni.nimh.nih.gov/pub/dist/doc/htmldoc/programs/3dLMEr_sphx.html#ahelp-3dlmer) (Chen, Saad, Britton, Pine, & Cox, 2013) in AFNI (Analysis of Functional NeuroImages, https://afni.nimh.nih.gov/) (Cox, 1996) was used to build the model and to perform the linear mixed-effect analysis. The model includes variates of sleep stages (including wakefulness), sessions, groups, age, sex and years of education. Stages and sessions were modeled as within-subject factors; participant groups were modeled as between-subject factors. Group-by-stage interaction and main effect of group were tested. We used the autocorrelation function (ACF) modeling approach combined with a voxelwise threshold of $p < .001$ to correct for multiple comparisons (Cox, 1996; Eklund, Nichols, & Knutsson, 2016). ACF was developed and implemented into the 3dClustSim tool to determine the cluster-size threshold to use for a given voxelwise threshold. ACF was estimated using 3dFWHMx based on the preprocessed fMRI data prior to functional connectivity calculations. Correspondingly, a corrected significance level of $p < .05$ for the resulting statistical maps was obtained

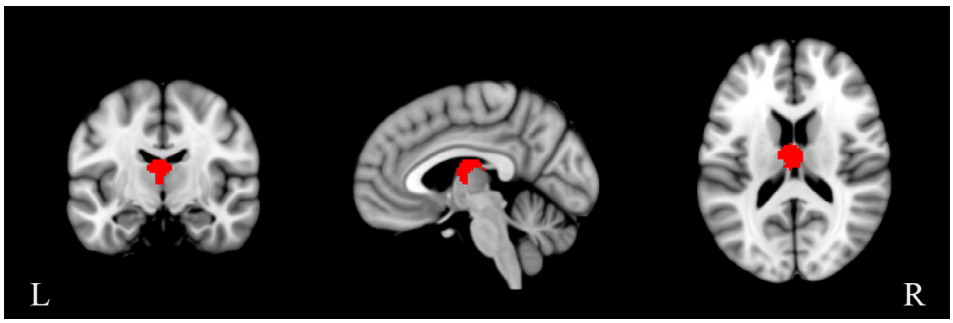

FIGURE 1 Thalamic seed selected on the basis of the Power264 template (Power et al., 2011). Slice coordinates: $X = -4$, $Y = -11$, $Z = 16$; L: left; R: right

using clusters, with a minimum number of 198 voxels at an uncorrected individual voxel height threshold of $p < .001$.

2.7 | Exploratory correlation analysis

The relationships between thalamic connectivity of the regions that showed a significant interaction or group effect and sleep-related clinical measurements, including the arousal index, awakening index (number of awakenings during sleep period time [SPT] divided by SPT), misperception index ((subjective total sleep time [TST] – objective TST)/objective TST) (Manconi et al., 2010), SDS and SAS were explored. The arousal index and awakening index to some extent indicate sleep disturbance and represent arousal levels during sleep, while the misperception index is suggested to be related to enhanced information processing caused by hyperarousal (Riemann et al., 2010). Notably, the participants with only one night of PSG recording and participants without a sleep diary of the second PSG night were not included in the correlation analyses. The misperception index derived from the EEG-fMRI night was also calculated to validate the PSG night data.

3 | RESULTS

3.1 | Demographic and clinical characteristics

The demographic and clinical characteristics of the 64 subjects are shown in Table 1. PSG-related clinical characteristics were derived from the PSG data of the second night, except for the three participants who had only one-night PSG recording. Subjective TST was derived from the sleep diary of the corresponding night. The first PSG nights were designed for reducing the first-night effect; hence, the data of the first PSG night were not analyzed here.

3.2 | Five-minute epochs of fMRI data

For the insomnia disorder group, there were 104 wakefulness epochs (17 subjects), 27 N1 epochs (13 subjects), 67 N2 epochs (20 subjects), and 20 N3 epochs (seven subjects). For the healthy control group, there were 40 wakefulness epochs (13 subjects), 16 N1 epochs (11 subjects), 75 N2 epochs (21 subjects), and 91 N3 epochs

TABLE 1 Demographic and clinical characteristics of the participants

Variable	Insomnia ($N = 33$)	Control ($N = 31$)	t	p
Age (year)	39.18 ± 9.42	35.06 ± 8.55	1.83	.072
Male/female	14/19	15/16	$\chi^2 = 0.23$	.632
Years of education	16.82 ± 2.93	16.61 ± 2.16	0.32	.752
TIB (min)	462.73 ± 64.68	481.53 ± 31.64	−1.44	.154
SPT (min)	441.52 ± 61.66	472.08 ± 34.31	−2.42	.019[a]
SOL (min)	14.79 ± 23.65	6.73 ± 5.76	1.85	.070
Objective TST (min)	400.23 ± 63.69	447.34 ± 40.15	−3.51	<.001[a]
SE (%)	86.84 ± 8.89	92.85 ± 5.24	−3.27	.002[a]
N1 (min)	36.51 ± 14.14	36.94 ± 11.58	−0.13	.897
N2 (min)	212.79 ± 44.57	238.94 ± 27.60	−2.80	.007[a]
N3 (min)	69.50 ± 29.13	71.68 ± 27.90	−0.31	.761
REM	81.42 ± 21.48	99.79 ± 21.39	−3.43	.001[a]
Awakening index (hr^{-1})	0.033 ± 0.013	0.029 ± 0.012	1.37	.176
Arousal index (hr^{-1})	6.29 ± 3.12	4.88 ± 1.87	2.17	.033[a]
Subjective TST (min)[b]	340.16 ± 103.40	455.75 ± 49.06	−5.44	<.001[a]
Misperception index[b]	−0.15 ± 0.20	0.02 ± 0.08	−4.26	<.001[a]
AHI (hr^{-1})	1.33 ± 1.24	0.91 ± 0.87	1.59	.118
PLMA (hr^{-1})	3.02 ± 4.47	2.65 ± 3.66	0.36	.717
PSQI	11.45 ± 3.26	2.32 ± 1.25	14.61	<.001[a]
SDS standard score	40.76 ± 8.30	30.32 ± 4.74	6.12	<.001[a]
SAS standard score	38.33 ± 6.87	28.39 ± 4.81	6.67	<.001[a]

Note: Mean ± *SD* of variables, *t*-statistics, and *p*-values are reported.

Abbreviations: AHI, apnea-hypopnea index; PLMA, periodic limb movements with awakening; PSQI, Pittsburgh sleep quality index; SAS, self-rating anxiety scale; SDS, self-rating depression scale; SE, sleep efficiency (objective TST/TIB); SOL, sleep onset latency; SPT, sleep period time; TIB, time in bed; TST, total sleep time.

[a]Significant difference between two groups ($p < .05$).

[b]The number of participants used to calculate these terms was 31 for the insomnia disorder group and 26 for the healthy control group due to the loss of the sleep diary for the PSG night for several participants.

(17 subjects). The detailed epoch distribution, TST and misperception index for the EEG-fMRI night are shown in Table S2. Ultimately, data from all 33 participants in the insomnia disorder group and 31 out of 32 in the healthy control group were included. Data from one healthy subject had no continuous 5-min epoch in any stage.

3.3 | Functional connectivity

A group-by-stage interaction was observed in seven clusters widely spread across cortical regions, including the bilateral occipital gyrus, temporal gyrus, frontal gyrus and right parietal gyrus (Figure 2a, Table 2). The average connectivity in these clusters is shown in Figure 2b. Post hoc analyses demonstrated that thalamic connectivity with these clusters was higher in the insomnia disorder group than in the healthy control group during wakefulness but lower during sleep. Furthermore, regarding the main effect of group, patients with insomnia disorder demonstrated decreased thalamic connectivity in the left putamen, parahippocampal gyrus, amygdala, pallidum, and hippocampus (Figure 3a, Table 2). This decrease was significant across all four stages (Figure 3b). A smaller cluster in the symmetric region was marginally significant (Table 2).

3.4 | Correlation analysis

Significance for correlation analysis was determined as $p < .0025$ (0.05/20 for Bonferroni correction considering five clinical parameters and four stages) for each cluster. The thalamic connectivity in the right temporal cluster (Table 2, Figure 2) and the limbic cluster (Table 2, Figure 3) in N1 was significantly correlated with the misperception index (Figure 4). No other significant correlation was found. Of note, the two significant correlations were not replicated using the misperception index derived from the EEG-fMRI nights.

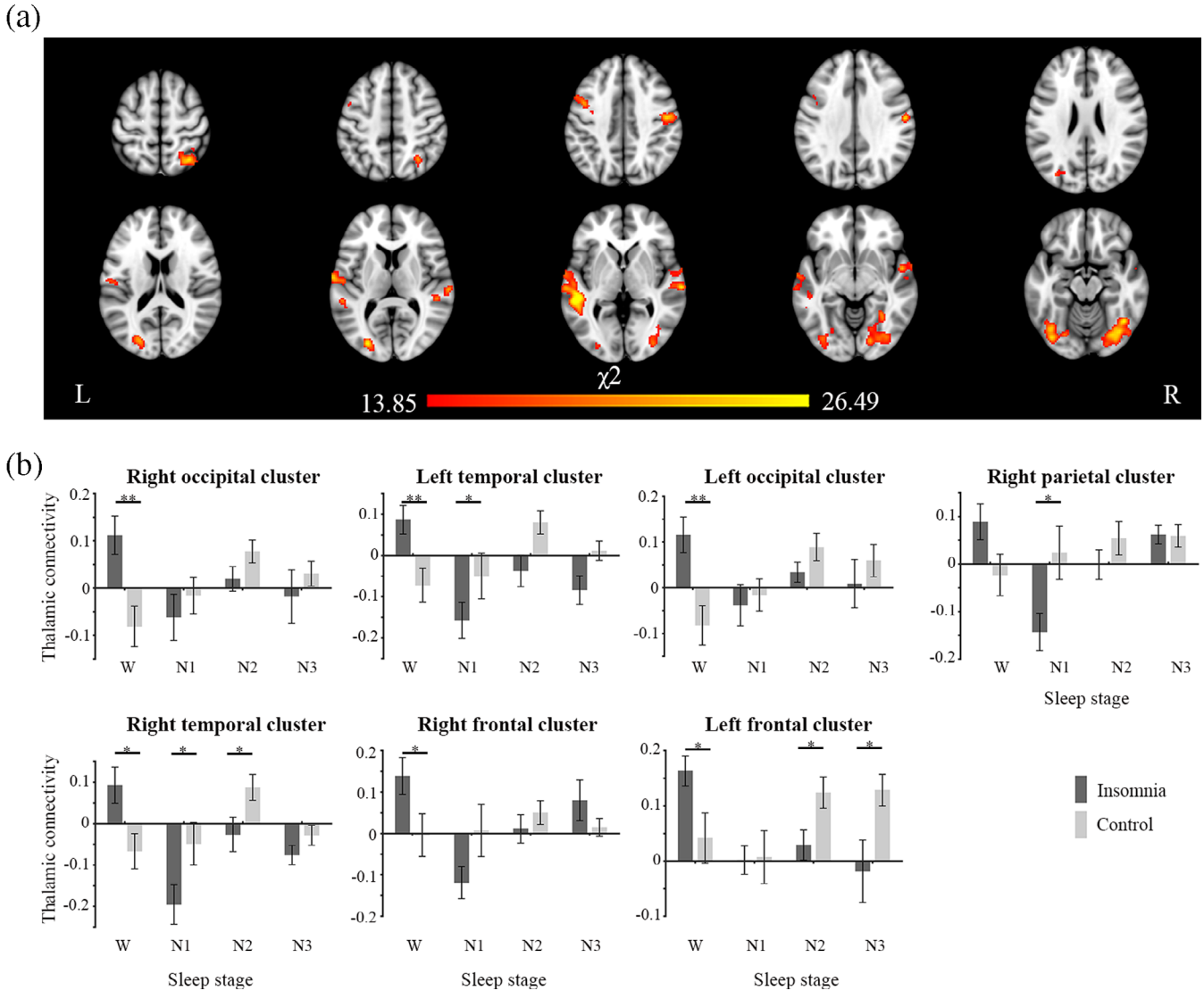

FIGURE 2 Group-by-stage interaction of thalamic connectivity. (a) Brain regions with a group-by-stage interaction ($p < .001$ and clusterwise corrected $p < .05$; slice coordinate: first slice: $Z = 57$, last slice: $Z = -15$, interval: 8; L: left, R: right). (b) Average Fisher z-values (mean ± *SEM*) in the clusters for the interaction shown in (a) (post hoc two-sample t-tests between the two groups, $^{*}p < .05$, $^{**}p < .01$)

TABLE 2 Altered connectivity with the thalamus in patients with insomnia disorder compared with healthy controls

Brain regions	MNI coordinates (*x*, *y*, *z*)	# of voxels	$\chi^2(2)$
Group-by-stage interaction			
FFG_R/LING_R/MOG_R/IOG_R/ITG_R	36, −70, −18	1,417	30.94
STG_L/MTG_L	−54, −36, 4	1,183	34.46
MOG_L/Fusiform_L/IOG_L/Cuneus_L	−38, −68, −16	923	28.53
SPG_R	36, −62, 62	630	26.17
STG_R/MTG_R	66, −22, 4	566	26.33
Precentral_R/Postcentral_R	56, −14, 36	286	27.50
Precentral_L/MFG_L	−42, 2, 40	200	23.66
Group effect			
PHG_L/HIP_L/PUT_L/AMYG_L/PAL_L	−20, −12, −14	577	25.84
AMYG_R/PUT_R/PHG_R	26, −2, −10	168	25.39

Abbreviations: AMYG, amygdala; FFG, fusiform gyrus; HIP, hippocampus; IOG, inferior occipital gyrus; ITG, inferior temporal gyrus; L, left; LING, lingual gyrus; MFG, middle frontal gyrus; MOG, middle occipital gyrus; MTG, middle temporal gyrus; PAL, pallidum; PHG, parahippocampal gyrus; PUT, putamen; R, right; SPG, superior parietal gyrus; STG, superior temporal gyrus; $\chi^2(2)$, peak chi-square value with two degrees of freedom.

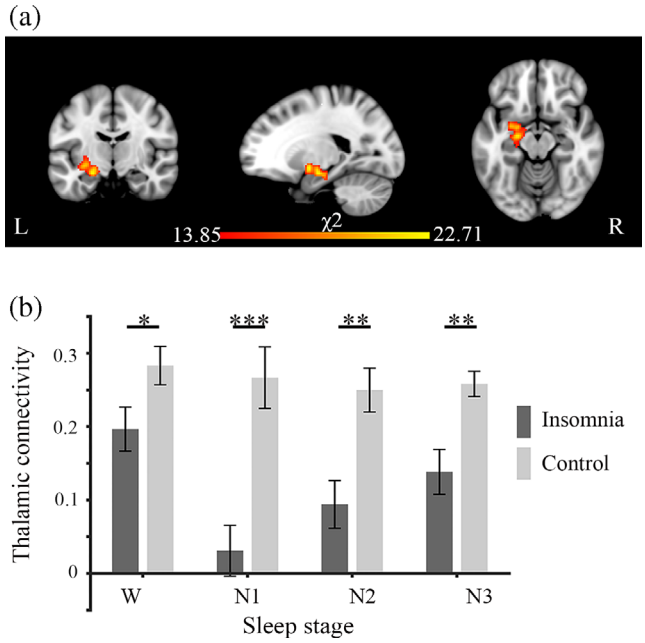

FIGURE 3 Main effect of group of thalamic connectivity. (a) Brain regions with a main effect of group ($p < .001$ and clusterwise corrected $p < .05$; slice coordinate: $X = -20$, $Y = -12$, $Z = -14$; L: left, R: right). (b) Average Fisher *z*-values (mean ± *SEM*) in the limbic cluster with a group effect as shown in (a) (post hoc two-sample *t*-tests between the two groups, $^*p < .05$, $^{**}p < .01$, $^{***}p < .001$)

3.5 | Additional analyses

Using thalamic seeds adopted from gray matter voxels (Figure S2) and from ICA (Figure S3), we observed also identical findings (Tables S3 and S4, Figures S4–S9).

4 | DISCUSSION

Using simultaneous EEG-fMRI, this study identified the thalamus-based brain functional networks across wakefulness and all NREM stages in patients with insomnia disorder. With this advantage, we illustrated altered thalamus-based connectivity in patients with insomnia disorder. Importantly, this study demonstrated that thalamic-limbic and thalamic-temporal connectivities are significantly correlated with sleep misperception derived from PSG data, providing possible explanations for the formation of sleep misperception.

The altered connectivity between the thalamus and cortical regions in patients with insomnia disorder provided possible brain functional network evidence for the hyperarousal hypothesis. Patients with insomnia disorder showed a more positive thalamocortical connectivity than the healthy controls during wakefulness. It was found that greater sleepiness was associated with lower or more negative thalamocortical connectivity (Killgore et al., 2015). The more positive thalamocortical connectivity indicated a more vigilant state of the brain of patients with insomnia disorder during night-time wakefulness. After falling asleep, the thalamocortical connectivity for the patients with insomnia disorder was lower (more negative) than that of the healthy controls. We previously found activation in the thalamus and deactivation in the temporal, occipital and frontal gyri during spontaneous arousals in NREM sleep (Zou et al., 2020). In addition, activation in the thalamus and deactivation in cortical regions have been found in studies using auditory and median-nerve stimuli during sleep (Czisch et al., 2004; Del Felice, Formaggio, Storti, Fiaschi, & Manganotti, 2012). Taken together, the evidence suggests that the anticorrelation between the thalamus and certain cortical regions during sleep is related to arousal level. This result indicated that patients with insomnia disorder were likely to be more disturbed and at a higher arousal level than healthy controls during sleep, especially light sleep.

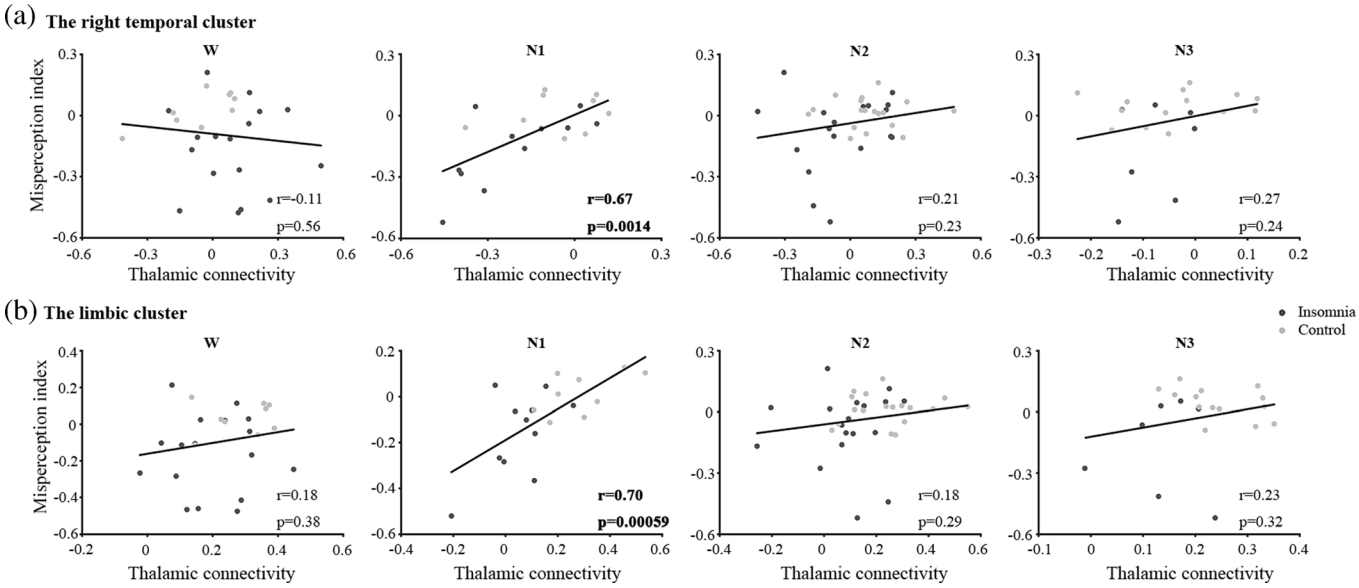

FIGURE 4 Association between the misperception index and thalamic connectivity in (a) the right temporal cluster, and (b) the limbic cluster

The trait-like decreases in connectivity between the thalamus and subcortical regions, including the hippocampus, amygdala, parahippocampal gyrus and lentiform nucleus, were consistent with the findings of abnormal activity in the amygdala and hippocampus in insomnia disorder. Abnormal activities of the amygdala have been found in patients with insomnia disorder (Baglioni et al., 2014; Huang et al., 2012; Wassing et al., 2019). Functional and structural changes in the hippocampus have also been identified (Joo, Kim, Suh, & Hong, 2014; Riemann et al., 2007). Patients with insomnia disorder have more negative emotions than good sleepers based on self-report, and dysfunction in emotional reactivity may modulate the relationships between insomnia and mood disorders, such as depression and anxiety (Baglioni, Spiegelhalder, Lombardo, & Riemann, 2010). We did not find a significant correlation between the thalamo-limbic connectivity and SDS or SAS. However, the altered thalamo-limbic connectivity added evidence that the activity was abnormal in the limbic system, which may be responsible for the prognosis between insomnia disorder and mood disorders.

Discrepancy between objective and subjective sleep parameters is a major clinical symptom in insomnia disorder (Bastien et al., 2014; Harvey & Tang, 2012). A previous study showed that cognitive and physiological arousal had effects on sleep perception (Tang & Harvey, 2004). It has been indicated that enhanced information processing may distort the distinction between sleep and wakefulness (Riemann et al., 2010). The altered network found in this study provided a possible explanation for the discrepancy between objective and subjective sleep perception. The amygdala, hippocampus and parahippocampal gyrus are involved in mood regulation and memory consolidation. The connectivity in N1 among these clusters was closely related to the misperception index (Figure 4b), indicating that the decrease in connectivity between the thalamus and these limbic regions during N1 affected the processing of circadian timing information. The misperception index is also correlated with connectivity in the temporal region (Figure 4a). The negative thalamic-temporal connectivity in N1 indicated that patients with insomnia disorder were more involved in information processing, which affected the processing of circadian timing information and resulted in sleep misperception. The correlations between thalamic connectivity and the misperception index were not replicated using the misperception index derived from the EEG-fMRI night. There was a much larger variance in the misperception index for both groups, and participants tended to overestimate their TST during the EEG-fMRI scan at an average level (Table S2). This may be because participants commonly woke up at midnight for EEG-fMRI; thus, their subjective report of TST varied from the morning report after the PSG night. In addition, the objective sleep quantity and quality would be affected during EEG-fMRI in a noisy and narrow environment.

The combination of fMRI and EEG is an appropriate method to investigate insomnia disorder since it can provide insights within specific stages (Spiegelhalder, Regen, Baglioni, Riemann, & Winkelman, 2013). The existing literature on fMRI studies in insomnia disorder has mainly investigated brain mechanisms of insomnia disorder during tasks and wakefulness, and has not revealed consistent results (Tahmasian et al., 2018). It is necessary to study brain activity during sleep for such a sleep disorder. To our knowledge, this study is the first to use simultaneous EEG-fMRI settings and disentangle the effect of physiological states on the functional networks in insomnia disorder. With this advantage, we found stage-specific alterations. The decrease in thalamo-limbic connectivity was most significant in N1, and correlations between thalamic connectivity and TST misperception were found in N1. A possible explanation for the peculiarity of N1 is that N1 is the lightest and most unstable sleep stage, making it the easiest to mistake as wakefulness by patients with insomnia disorder. Abnormal sensory information involvement and

loss of memory and mood regulation regarding circadian information during N1 may cause sleep misperception in insomnia disorder. However, the duration of N1 (less than 40 min, Table 1) cannot explain all of the subjective-objective TST differences (approximately 60 min, Table 1). Evidence has been found that rapid eye movement (REM) sleep contributes to sleep misperception (Feige et al., 2008). REM sleep has similar EEG characteristics to N1, and the brain functional networks during REM should be investigated in future studies. The current findings suggested certain brain regions as potential therapeutic targets and identified N1 as an important physiological state for insomnia disorder treatments.

There are certain limitations in this study. First, due to the intrinsic characteristics of night-time EEG-fMRI experiments and our stringent rule regarding sleep stage scoring (consistency between two raters), the sample size was relatively limited and unbalanced. Some subjects had only one 5-min epoch in certain sleep stages, which may affect the reliability of functional connectivity (Noble, Scheinost, & Constable, 2019). To increase the within-subject data to improve reliability, a longer recording time is needed. A full-night scanning pulse sequence can be helpful for future studies (Moehlman et al., 2019). Second, we recorded data only from the early portion of the sleep period because of the technical restriction of the pulse sequence and the compliance of the participants. We could restart another EEG-fMRI scan, but the obvious changes in scanner noise during the switch between fMRI scans would have aroused the subjects and affected the sleep structure. However, in future studies, this problem can be solved by modifying the pulse sequence to overcome limits related to scanning volume in a session (Moehlman et al., 2019). With this advantage, data on REM stage can also be recorded, and the brain mechanisms of insomnia disorder can be more comprehensively understood. Third, certain covariates were not perfectly controlled. We did not record peripheral pulse units or respiratory belt signals, considering it could make it harder to fall asleep. We applied CompCor to regress out the nuisance signals. However, there may still be a residual physiological noise, which can have a baseline difference in metabolism for the two groups due to hyperarousal in the insomnia disorder group. Though CompCor could remove physiological noises to a large extent, future studies should pay more attention to controlling physiological noises in the insomnia study, and the influence of such physiological nuisances on fMRI studies of insomnia needs to be investigated. In addition, there was a relatively large difference in age between the two groups ($p = .07$, Table 1). Studies have found that sleep structures change with age, and sleep quality generally decreases with age (Ohayon, Carskadon, Guilleminault, & Vitiello, 2004; Redline et al., 2004). Though age was taken into account as a covariate in the modeling, it might still confound the findings.

5 | CONCLUSIONS

Simultaneous EEG-fMRI data were recorded and analyzed across wakefulness and all NREM stages in patients with insomnia disorder. We illustrated altered thalamus-based connectivity in patients with insomnia disorder. Abnormal connectivity in N1 may underlie the mechanism of sleep misperception in insomnia disorder. Our findings shed light on the brain mechanisms of insomnia disorder and provide potential therapeutic targets for the treatment of insomnia disorder. More studies regarding the sleep state of patients with insomnia disorder should be conducted in the future.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

As per regulation of the institutional review board of Peking University, Beijing, China, the fMRI data are unsuitable for public deposition. Nevertheless, researchers interested in accessing the data may contact the corresponding author, who will help in the bureaucratic procedures to forward the request to the institutional review board of Peking University.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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