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Acupuncture modulates emotional network resting-state functional connectivity in patients with insomnia disorder: a randomized controlled trial and fMRI study

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Abstract

Background Insomnia disorder (ID) is one of the most common sleep problems, usually accompanied by anxiety and depression symptoms. Functional magnetic resonance imaging (fMRI) study suggests that both poor sleep quality and negative emotion are linked to the dysregulation of brain network related to emotion processing in ID patients. Acupuncture therapy has been proven effective in improving sleep quality and mood of ID patients, but the involved neurobiological mechanism remains unclear. We aimed to investigate the modulation effect of acupuncture on resting-state functional connectivity (rsFC) of the emotional network (EN) in patients experiencing insomnia.

Methods A total of 30 healthy controls (HCs) and 60 ID patients were enrolled in this study. Sixty ID patients were randomly assigned to real and sham acupuncture groups and attended resting-state fMRI scans before and after 4 weeks of acupuncture treatment. HCs completed an MRI/fMRI scan at baseline. The rsFC values within EN were calculated, and Hamilton Anxiety Scale (HAMA), Hamilton Depression Scale (HAMD), Pittsburgh Sleep Quality Index (PSQI), Hyperarousal Scale (HAS), and actigraphy data were collected for clinical efficacy evaluation.

Results Resting-state FC analysis showed abnormalities in rsFC centered on the thalamus and dorsolateral prefrontal cortex within EN of ID patients compared to HCs. After real acupuncture treatment, rsFC of the anterior cingulate cortex, hippocampus, and amygdala were increased compared with the sham acupuncture group ($p < 0.05$, FDR corrected). In real acupuncture group, the rsFC value was decreased between left amygdala and left thalamus after 4 weeks of treatment compared with baseline. A trend of correlation was found that the increased rsFC value between the right amygdala and left hippocampus was positively correlated with the decreased HAMA scores across all ID patients, and the decreased left amygdala rsFC value with the left thalamus was negatively correlated with the increased sleep efficiency in the real acupuncture group.

Conclusion Our findings showed that real acupuncture could produce a positive effect on modulating rsFC within network related to emotion processing in ID patients, which may illustrate the central mechanism underlying acupuncture for insomnia in improving sleep quality and emotion regulation.

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Keywords Insomnia disorder, Acupuncture, Emotional network, Resting-state functional connectivity (rsFC), Functional magnetic resonance imaging (fMRI)

Introduction

As the most frequently encountered sleep affliction, insomnia disorder (ID) is characterized by difficulties in initiating sleep or waking up during the night or earlier in the morning than one would like [1]. Approximately 22.1% of the general population suffer from chronic insomnia [2], and most of them experience persistent negative moods such as anxiety and depression [3]. The coexistence of insomnia and negative emotions will prolong the course of disease and reduce the quality of life in patients [4].

It is believed that insomnia and negative emotions have partially similar pathophysiological pathways [5]. Hyperarousal is widely accepted as a hypothesis to explain the pathogenesis of insomnia, meaning a state of excessive arousal of cognition, physiology, and cerebral cortex across the sleep-wake cycle [6]. Excessive cortical arousal can lead to emotional rumination and anxiety [7, 8]. The constant worry about the inability to sleep causes mental hyperarousal, leading to the vicious circle of insomnia and anxiety [5, 9]. Additionally, functional magnetic resonance imaging (fMRI) studies have shown that insomnia, anxiety, and depression are all linked to dysregulation of emotion-related regions, further demonstrating a close relationship between insomnia and negative emotion [10, 11].

It has been proven that emotional management could be helpful in improving sleep quality [12]. Although cognitive behavioral therapy (CBT) and pharmacotherapy are standard therapies for treating ID, the high cost and inconsistent efficacy of CBT and the dependence on medications hinder the effectiveness of treating ID [13, 14]. Acupuncture, as a complementary therapy, has been proven effective in the therapy of insomnia and some psychological disorders [15, 16]. Acupuncture is valuable for improving sleep quality [17], decreasing hyperarousal [18], relieving anxiety [19] and depression [20] in patients with ID. Acupuncture has the advantages of being safe, affordable, and effective [21]. However, the neurological mechanism underlying the effect of acupuncture remains unclear.

FMRI studies have shown abnormal neural activity of brain regions underlying emotional reception and expression in ID patients. In general, these emotional related areas are usually concentrated in the amygdala, anterior cingulate cortex (ACC), thalamus, hippocampus and prefrontal cortex (PFC) [22, 23], which are collectively considered as the emotional network (EN) [23, 24]. Dysregulation of the EN has been demonstrated to be associated with the occurrence of insomnia [25, 26]. Klumpp et al. found that worse sleep quality corresponded with increased amygdala-ACC functional connectivity [27]. Kim et al. discovered increased FC between the thalamus and cortical areas in the frontal, parietal, and temporal lobes in ID patients, and the FC values were negatively correlated with sleep efficiency [17]. Furthermore, Zhu et al. and Sanford et al. proved that the FC value between ACC and insula was closely related to the severity of anxiety and the FC of PFC was involved in sleep and cognition regulation [28, 29].

Recently, acupuncture has been shown to have a positive effect on regulating regional functional activity in hippocampus [30], amygdala, ACC [31], PFC [32, 33] and thalamus [34] in ID patients. Therefore, we speculate that modulation of functional activity in the emotion-related areas might be the potential mechanism of acupuncture's effect in relieving negative mood and insomnia. However, there is a lack of research on the modulation of EN in insomnia patients by acupuncture.

Resting-state FC analysis provides temporal correlation between discrete or continuous timeseries of brain regions [35]. Investigation of brain rsFC would be helpful for a comprehensive understanding of the synchronization among different brain regions [36], which could reveal changes in brain networks in psychiatric mental illness and insomnia [37, 38]. In this study, we aimed to investigate the changes of rsFC in the EN regions in patients with insomnia modulated by acupuncture.

Materials and methods

This trial was performed at the Beijing Hospital of Traditional Chinese Medicine, Capital Medical University (Beijing TCM Hospital). It was approved by the ethics committee of the hospital and registered on http://www.chictr.org.cn (Chinese Clinical Trials Register, ChiCTR1800015282, 20/03/2018). The protocol of this study has been published previously [39].

Participants

According to the Diagnostic and Statistical Manual of Mental Disorders (5th edition) (DSM-5), ID patients were recruited from outpatient acupuncture clinics of Beijing TCM Hospital from September 2019 to

September 2021. All patients with ID were required to sign an informed consent. Insomnia was diagnosed based on the DSM-5 by Jing Guo, a specialist working at the neurology outpatient service of the Beijing TCM Hospital. In addition, we also recruited 30 age-, education-, and sex-matched healthy participants from the local community to serve as the control group through advertising posters.

The inclusion criteria of ID patients were as follows: (1) age 18–60 years; (2) Pittsburgh Sleep Quality Index (PSQI) score > 8 [40]; (3) Hamilton Depression Scale (HAMD) score < 7 [41]; (4) Hamilton Anxiety Scale (HAMA) score < 14 [42]; (5) Hyperarousal Scale (HAS) score > 32 [43]; (6) informed consent; (7) patients not taking anti-anxiety, anti-depressant, or sleep medication in the past month; (8) right-handedness. ID patients were excluded from the study if any of the following criteria were met: (1) depression, severe anxiety, schizophrenia, or other serious mental illnesses; (2) severe heart, brain, kidney, or liver disease; (3) failure to cooperate with the examination and treatment; (4) apnea syndrome; pregnant and lactating women; (5) claustrophobia or other contraindications to MRI examination; 7) and a definite lesion on MRI or grossly asymmetrical anatomy of the head. Healthy controls (HCs) were included in the study according to the following criteria: (i) they had good sleep habits and PSQI score < 3; (ii) HAMA score < 7; (iii) Hyperarousal Scale (HAS) score < 32; (iv) no serious heart, lung, kidney, or neuropsychological disease; (v) no structural abnormalities on conventional brain magnetic resonance imaging (MRI); (vi) females were not pregnant or nursing; and (vii) right-handedness.

Experimental design

The study is a randomized, single-blind, controlled clinical trial. ID patients were included and randomly assigned to the real acupuncture group and the sham acupuncture group in a 1:1 ratio, using opaque, sequentially numbered sealed envelopes. All ID patients were blinded to their group allocation. Due to the feature of acupuncture, the acupuncturist was not blinded to patients' allocation; however, ID patients and acupuncturist are prohibited from discussing group allocation. To comply with the single-blind principle, assessors and statisticians involved in data collection and analysis were blinded to the assignments.

Acupuncture treatment

In the trial, all acupuncture procedures were performed by a licensed acupuncturist with 20 years of experience. All ID patients in the real and sham acupuncture groups received 12 sessions of acupuncture treatment in 4 weeks with different acupoint prescriptions and manipulation, with 3 sessions per week.

ID patients in real acupuncture group received manual acupuncture in Baihui (DU20), Shenting (DU24), Sishencong (EX-HN1), bilateral Shenmen (HT7), bilateral Benshen (GB13), bilateral Neiguan (PC6), and bilateral Sanyinjiao (SP6) acupoints after skin disinfection using 75% alcohol. The positioning standard refers to *the National Standard for Acupuncture and Moxibustion Meridian Point Positioning* promulgated by the National Standard GB12346-90 of China. Details of more information on acupuncture (location of real and sham acupoints, depth of insertion) are shown in Figs. 1 and 2. The needles were twisted (bi-directionally, 90°–180°), lifted,

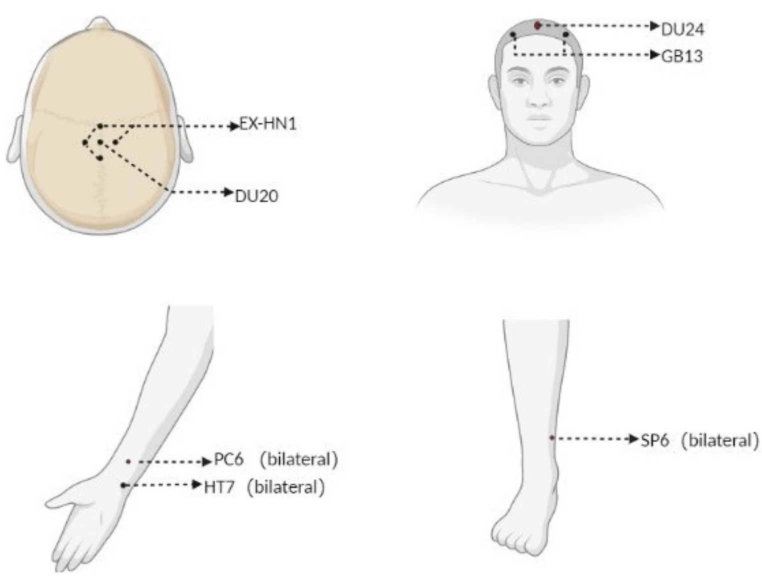

Fig. 1 Acupoints location of real acupuncture group

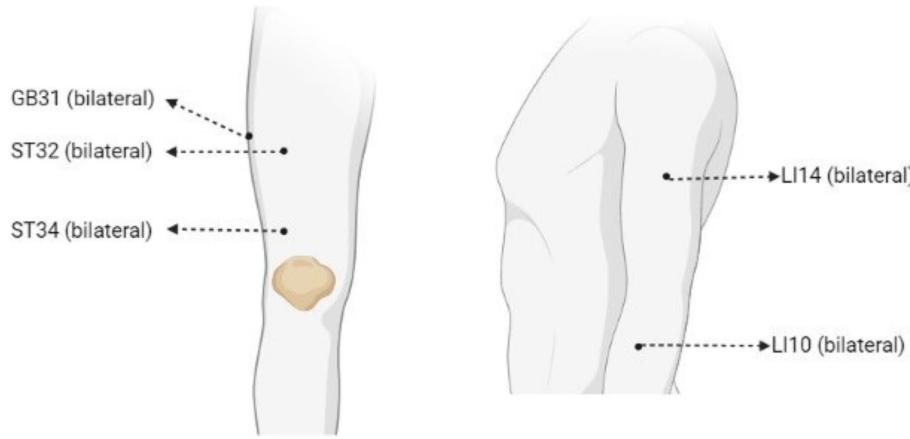

Fig. 2 Acupoints location of sham acupuncture group

and thrust (3–5 mm) by an acupuncturist to induce the Deqi sensation (distension, heaviness, and numbness sensation; the key to effect of acupuncture). ID patients were given 30 min of treatment time per session. Disposable sterile needles (0.25 × 25 mm, Huatuo Medical Instrument Co., ltd, Suzhou, China) were used in this study.

ID patients in sham acupuncture group received acupuncture in bilateral Binao (LI14), Shousanli (LI10), Fengshi (GB31), Futu (ST32), and Liangqiu (ST34). These acupoints had no therapeutic effect on insomnia according to literature search and previous research [44, 45]. The needles were inserted 1–2 mm vertically to penetrate superficial skin at these non-effective acupoints to avoid the sensation of Deqi. The sham control method is commonly used in randomized controlled trials of the efficacy of acupuncture [46]. ID patients were also given 30 min of treatment time per session. The frequency of treatments and needles was the same as that in the real acupuncture group.

If a subject suffers a serious adverse injury from needling during the experiment (e.g., severe local and systemic infection, significant vascular injury, puncture of organs and nerves), adverse events should be recorded in detail. All ID patients were given practical sleep guidance during the treatment. If necessary, ID patients were allowed to take zopiclone, and the dosage and times of administration should be recorded in detail.

Sample size estimate

In our previous pilot study [47], the PSQI score significantly decreased by 4.43 ± 3.60 in the acupuncture group and by 1.30 ± 2.58 in the control group. Using a t-test of two independent samples with uneven variance in PASS for calculation, the withdrawal rate is 20% to ensure that the results are statistically different ($\alpha = 0.05$, $1\text{-}\beta = 0.9$); therefore, each group enrolled 30 subjects.

Clinical assessment and data analysis

Sleep quality evaluation: PSQI

The primary outcome measure was the PSQI scale. The PSQI scale was developed by Dr. Buysse in 1989; it is the most widely used indicator for assessing sleep quality in insomniacs, including sleep duration, efficiency, depth, and abnormal sleep sensations [40]. The higher PSQI score, the poorer sleep quality. Reference of PSQI scores: 0 ~ 5 Sleep quality is very good; 6 ~ 10 Sleep quality is okay; 11 ~ 15 Average sleep quality; 16 ~ 21 Sleep quality is very poor. It was collected at baseline and after 4 weeks acupuncture treatment.

Emotion evaluation: HAMD and HAMA

The emotion evaluation outcomes were measured by HAMD and HAMA scales. The 17-items HAMD is a widely used rating scale to assess the severity of depression [41]. Reference of HAMD scores: 0 ~ 6 none or may have mild depressive symptoms but do not reach a diagnosis of depression disease [48]; 7 ~ 17 possible diagnosis of depressive disorder; 18 ~ 24 diagnosed depression; 25–52 major depression.

The 14-items HAMA was developed by Hamilton in 1959; it is one of the more widely used physician-rated scales to assess the severity of anxiety [42]. Each item has five possible answers with scores from 0 to 4. Reference of HAMA scores: 0 ~ 6 without anxiety symptoms; 7 ~ 14 may have anxiety; 15 ~ 21 definitely anxious; 22 ~ 29 obvious anxiety; 29 ~ 54 serious anxiety. HAMA and HAMD scales were collected at baseline and after 4 weeks acupuncture treatment.

HAS

The self-reported HAS is used to assess cognitive and emotional hyperarousal in response to stimuli during wakefulness [43]. It could measure symptoms of cortical hyperexcitability in ID participants [49]. The HAS consists of 26 items; each item is scored on a scale of 0–3, with higher scores indicating higher levels of cortical arousal. The total score above 32 indicates a state of hyperarousal [43]. HAS scale were collected at baseline and after 4 weeks acupuncture treatment.

Actigraphy

All of the ID patients were requested to wear an actigraphy unit (MTI Health Services Company, Pensacola, FL, USA) on the left wrist to detect sleep at night. Actigraphy, a wearable sleep device for sleep quality monitoring, is an objective indicator that reflects sleep time and quality in ID patients [50]. Actigraphy data were collected at night for one week before the first acupuncture intervention and for one week after the last acupuncture intervention. The total sleep time (TST), wake after sleep onset (WASO), and sleep efficiency (SE) were recorded to evaluate the objective sleep status of ID patients.

Clinical data analysis

Statistical evaluation of the clinical data was performed using SPSS software V21.0 (IBM SPSS Statistics, IBM Corp, Somers, NY). T-test was used for continuous measurement data conforming to normal distribution, and the Wilcoxon rank sum test was used for data not conforming to normal distribution. We set statistical significance at $p < 0.05$, with two-tailed analysis. The continuous variables that conform to normal distribution are presented as mean ± standard deviation (SD), those that are not conform to normal distribution are presented as median and interquartile range. One-way analysis of variance (ANOVA) was used for age between HCs and the real and sham acupuncture groups. For the sex and education level, we used the chi-square test to compare the dichotomous data. All ID patients were required to complete a questionnaire about their demographic characteristics and clinical questionnaires before treatment and after 4-weeks acupuncture treatment. Clinical data statistics followed the intention-to-treat (ITT) principle [51], and the abscission of clinical data was replaced by the group mean or median.

fMRI image acquisition

All MRI/fMRI scans were conducted on a Siemens 3.0 Tesla MRI scanner (Siemen Magnetom Verio, Erlangen, Germany) with a 24-channel phased-array head coil at Dong Zhimen Hospital Beijing University of Chinese Medicine. All participants underwent whole-brain conventional structural imaging and resting-state fMRI scans. They were required to stay awake and relaxed, remain motionless, and keep their eyes open during the scan. All MRI/fMRI scans were conducted between 9:00 am and 11:30 am. High-resolution brain T1-weighted brain anatomical images were collected using a volumetric three-dimensional magnetization prepared by a rapid-acquisition gradient-echo (MPRAGE) sequence with the following parameters: repetition time (TR) = 2000 ms, echo time (TE) = 3.51 ms, flip angle (FA) = 7°, slice number = 188, slice thickness/gap = 1.0/0 mm, voxel size = 1.0 mm × 1.0 mm × 1.0 mm, matrix = 256 × 256, and field of view (FOV) = 256 mm × 256 mm. The rs-fMRI images were obtained using an echo-planar imaging (EPI) sequence, and the parameters are as follow: TR = 2000 ms, TE = 30 ms, FA = 90°, slice number = 32, slice thickness/gap = 3.5/0.6 mm, voxel size = 3.5 mm × 3.5 mm × 3.5 mm, FOV = 224 mm × 224 mm, matrix size = 64 × 64, and phase-encoding direction: Anterior > > Posterior. In total, 240 volumes were acquired, and the EPI sequence scan time was 8 min. Each ID patient attended two MRI/fMRI scan visits before and after 4 weeks of acupuncture treatment. HCs completed an MRI/fMRI scan at baseline.

Image pre-processing and analysis

Image pre-processing

The fMRI data pre-processing and analysis were performed with DPABI V5.1_201201 (5.1, advanced edition, https://www.rfmri: dpabi) [52] within MATLAB_R2015b (Mathworks, Inc., Natick, MA). First, we converted the raw data from EPI DICOM format to NIFTI format before preprocessing. Then, the data were preprocessed as follows: (1) the first 10 time points were removed; (2) slice timing was performed to correct for timing differences between slices; (3) realignment to reduce motion-related variance, in which participants were excluded based on the criteria of translational movement > 3.0 mm and > 3.0° rotation [53]; (4) segmentation to split the image into gray matter, white matter and cerebrospinal fluid (CSF); (5) detrending and nuisance covariates regression procedures were preformed to remove linear trends and nuisance signals from the white matter, CSF, global signal time series; (6) the generated images were normalized to the standard template in the Montreal Neurological Institute (MNI) space; (7) low-frequency filtering (0.01–0.08 Hz) was performed to eliminate high frequency noises from the physiology; (8) to increase the signal-to-noise ratio of the image, spatial smoothing was analyzed using a [6] full-width at half maximum Gaussian kernel.

Resting-state FC analysis within emotional network

Resting-state FC analysis shows synchronization and cooperation of relevant brain regions [54]. Seed-based FC analysis was used for calculating FC value between specific region pairs within networks [55, 56]. The thalamus, ACC, DLPFC, hippocampus, and amygdala are mainly responsible for emotional management [22, 23]; thus, we predefined these brain regions as regions of interest (ROIs), a total of 10 ROIs in bilateral. The masks of 10 ROIs were extracted from the automated anatomical labeling (AAL) atlas using WFU_PickAtlas Toolbox V3.0.5 (https://www.nitrc.org/projects/wfu_pickatlas/). An ROI was considered a node of EN [57]. The representative BOLD time series of each ROI was calculated by averaging the regressed time series of all the voxels in the ROI mask. The edges of emotional network were determined by calculating the pairwise Pearson's correlations for all ROI pairs. In this way, a 10×10 correlation matrix of each participant was obtained. To follow normal distribution, the correlation coefficients were Z-standardized by Fisher's R-to-Z transformation.

Two sample t-test was used to compare the rsFC value between groups (HCs vs. ID patients at baseline, the real vs. the sham acupuncture group after 4 weeks of treatment), and paired *t*-test was used to compare within groups (pre- and post- treatment) using Graph Theoretical Network Analysis (GRETNA; http://www.nitrc.org/projects/gretna/), with head motion parameters, sex, age, education, and duration of insomnia as covariates. Network-based statistics (NBS) method was used to identify functional connectivity of brain network differences between real and sham groups, and the number of permutation tests was 1000 [58, 59]. The results were corrected with the False Discovery Rate (FDR, cluster-level $p < 0.05$).

Correlational analysis

Pearson's correlation analysis [60] was performed using SPSS V21.0 to investigate the relationship between rsFC changes and clinical data changes (including PSQI, HAMA, HAMD, HAS, TST, WASO, and SE). The Shapiro-Wilks test was used to check the normality of the data. We extracted the average z-score values of rsFC that had a significant difference between groups and within groups. Next, the relationship between Δ rsFC (post - pre) and Δ clinical outcomes (post - pre) was examined. Bonferroni corrections were used for multiple comparisons. Both uncorrected *p* value and corrected *p* value were reported. Hierarchical multiple regression analysis was applied to test the correlation between the mean z-score of changed rsFC and altered clinical data, using age, sex, education, and duration of insomnia as covariates. The significance level was set at $p < 0.05$.

Results

Participants and baseline demographics

A total of 30 HCs and 60 ID patients were recruited into the study and the 60 ID patients were randomly assigned to the real and sham acupuncture group in a 1:1 ratio. Ten ID patients were withdrawn, including four patients in real acupuncture group due to COVID-19 ($n = 3$) and time restriction ($n = 1$), six patients in sham acupuncture group due to COVID-19 ($n = 4$) and unsatisfactory effect ($n = 2$). Finally, fifty ID patients (26 in the real acupuncture group and 24 in the sham acupuncture group) completed the study. The procedure for the study is illustrated in Fig. 3. No patient reported the administration of hypnotic drug medications.

There was no significant difference in demographics between the HCs, the real acupuncture group, and the sham acupuncture group ($p > 0.05$) (Table 1). There was no significant difference in HAMA, HAMD, PSQI, HAS scores, TST, WASO, and SE between the real and sham acupuncture groups in the baseline period ($p > 0.05$), suggesting that the baseline conditions of the real and sham acupuncture groups were comparable (Table 2). Statistical significance was set at $p < 0.05$.

clinical results

After 4 weeks of treatment, the HAMA, HAMD, PSQI, and HAS scores decreased significantly in the real acupuncture group compared with the sham acupuncture group ($p < 0.05$). Actigraphy results showed that the real acupuncture group had longer TST and higher SE compared with the sham acupuncture group ($p < 0.05$); however, there was no statistically significant difference between the two groups in reducing the time of nighttime awakenings ($p > 0.05$) (Table 2). Neither the real nor the sham acupuncture group experienced serious adverse effects during acupuncture treatment.

Resting-state FC results

Baseline comparison between HCs and ID patients of rsFC within EN

In the baseline period, compared with HCs, ID patients showed increased rsFC values in right ACC-left DLPFC ($t = 2.045$, $p = 0.044$), right thalamus-left amygdala ($t = 2.907$, $p = 0.005$), left thalamus-left amygdala ($t = 3.170$, $p = 0.002$), left hippocampus-right thalamus ($t = 2.111$, $p = 0.038$), left hippocampus-left thalamus ($t = 2.439$, $p = 0.017$), and left DLPFC-right thalamus ($t = 2.247$, $p = 0.027$). Results are shown in Table 3; Fig. 4. Statistical significance was set at cluster-level $p < 0.05$ (FDR corrected).

Inter-group comparison of rsFC within EN

A total of fifty ID patients' fMRI images were acquired. Compared with sham acupuncture group, real acupuncture group showed increase rsFC values in right ACC-left ACC ($t = 2.881$, $p = 0.006$), left hippocampus-right ACC ($t = 2.066$, $p = 0.044$), right amygdala-left ACC ($t = 2.276$,

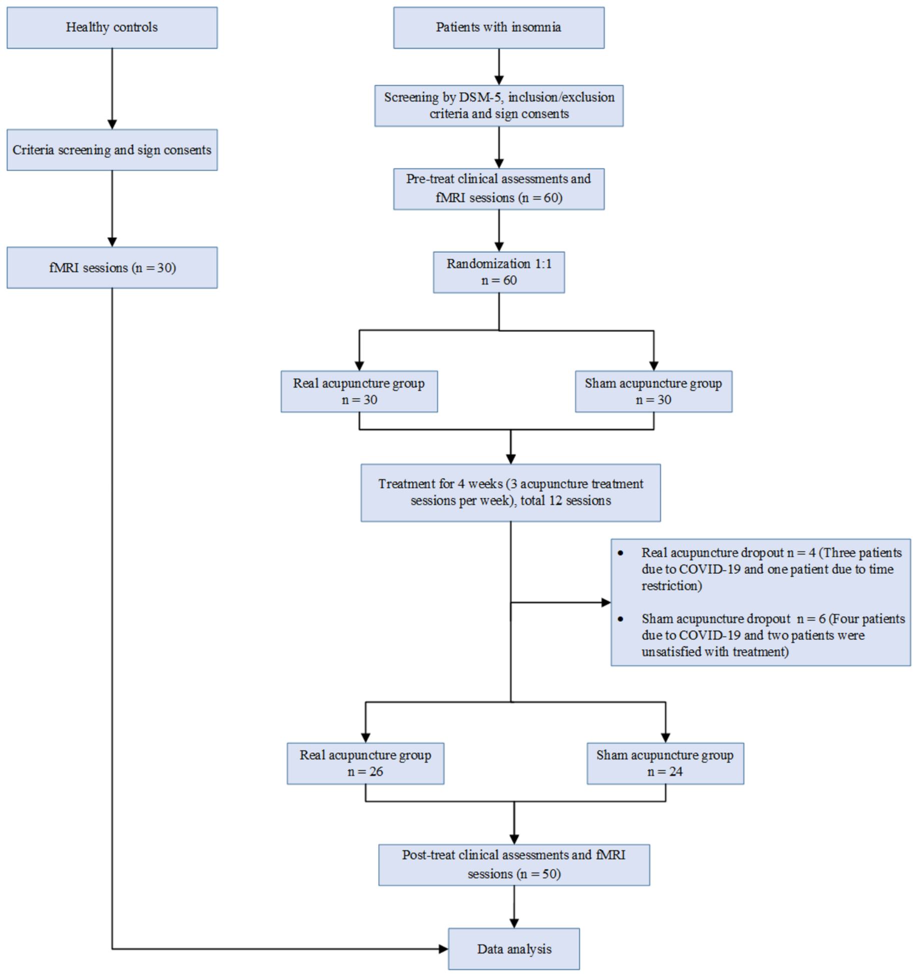

Fig. 3 Procedure for the study

Table 1 Demographics of participants

	Healthy controls ($n=30$)	Real acupuncture group ($n=30$)	Sham acupuncture group ($n=30$)	F/X^2/T value	*p* value
Age (mean ± SD)	35.23 ± 7.46	36.67 ± 2.13	37.50 ± 1.80	0.342[a]	0.712
Sex (male/female)	7/23	9/21	11/19	1.272[b]	0.529
Education (above/below bachelor's degree)	19/11	17/13	23/7	2.846[b]	0.241
Duration of insomnia (years, mean ± SD)	NA	4.54 ± 4.41	4.64 ± 5.22	0.082[c]	0.416

NA Not available

[a] One-Way ANOVA analysis (F value) was used for age of the three groups

[b] Chi-square analysis (X^2 value) was used for sex and education of the three groups

[c] Two sample t-test (T value) was used for the duration of insomnia between the real and sham acupuncture groups

$p=0.027$), left hippocampus-left ACC ($t=2.779$, $p=0.008$), left amygdala-right amygdala ($t=2.400$, $p=0.020$), and left hippocampus-right amygdala ($t=2.552$, $p=0.014$) after 4-weeks acupuncture treatment. Results are shown in Table 4; Fig. 5. Statistical significance was set at cluster-level $p<0.05$ (FDR corrected).

Intra-group comparison of rsFC within EN

Paired *t*-test was used to compare the rsFC of EN within groups before and after treatment. In real acupuncture group, the rsFC decreased in left amygdala-left thalamus after 4-weeks acupuncture treatment compared with baseline period ($t = -2.719$, $p=0.009$).

Table 2 Clinical data results in the intention-to-treat population

	Real acupuncture group ($n = 30$)	Sham acupuncture group ($n = 30$)	p value
HAMA Score [M (QL, QU)]			
Pre-treatment	12.00 (9.75 ~ 13.00)	12.00 (11.00 ~ 13.00)	0.511
Post-treatment	6.91 (3.00 ~ 11.00)	9.70 (8.75 ~ 10.25)	0.014*
Post - Pre	-5.00 (-7.00 ~ -1.75)	-2.00 (-3.15 ~ -1.00)	0.040*
HAMD Score [M (QL, QU)]			
Pre-treatment	6.00 (5.00 ~ 6.00)	6.00 (5.00 ~ 6.00)	0.084
Post-treatment	4.00 (3.00 ~ 6.00)	5 0.00(4.96 ~ 6.00)	0.000*
Post - Pre	-1.00 (-3.00 ~ 0.00)	-0.04 (-1.00 ~ 0.00)	0.000*
PSQI Score [M (QL, QU)]			
Pre-treatment	11.00 (10.00 ~ 14.00)	12.00 (11.00 ~ 14.00)	0.444
Post-treatment	7.00 (5.00 ~ 10.00)	11.16 (9.00 ~ 13.00)	0.000*
Post - Pre	-5.00 (-7.25 ~ -0.75)	-1.92 (-3.21 ~ 0.25)	0.001*
HAS Score [M (QL, QU)]			
Pre-treatment	44.00 (37.00 ~ 49.25)	44.00 (34.00 ~ 48.25)	0.673
Post-treatment	35.16 (27.25 ~ 42.25)	40.68 (33.50 ~ 46.50)	0.023*
Post - Pre	-10 (-15.25 ~ -4.00)	-2.66 (-6.00 ~ 1.25)	0.001*
TST [$\bar{x} \pm s$ (min)]			
Pre-treatment	394.55 ± 46.48	410.35 ± 54.08	0.660
Post-treatment	388.42 ± 59.67	382.49 ± 44.89	0.034*
Post - Pre	13.96 ± 62.60	-8.22 ± 37.27	0.101
WASO [$\bar{x} \pm s$ (min)]			
Pre-treatment	86.18 ± 60.06	86.90 ± 72.40	0.970
Post - treatment	60.45 ± 28.15	68.64 ± 28.12	0.264
Post - Pre	-29.24 ± 63.17	-15.08 ± 53.84	0.354
SE [$\bar{x} \pm s$ (%)]			
Pre-treatment	79.47 ± 6.22	79.15 ± 14.78	0.910
Post-treatment	87.58 ± 3.62	83.16 ± 6.26	0.001*
Post - Pre	8.61 ± 5.10	3.73 ± 13.34	0.066

The HAMA, HAMD, PSQI, and HAS scores did not conform to the normal distribution; the median (quartile) was used for statistical description; the Wilcoxon rank sum test of two independent samples was used between groups. The TST, WASO, and SE conformed to the normal distribution; means ± standard deviation ($\bar{x} \pm s$) was used for statistical description. Two-sample t-tests were used between groups

HAMA Hamilton Anxiety Scale, *HAMD* Hamilton Depression Scale, *HAS* Hyperarousal Scale, *PSQI* Pittsburgh Sleep Quality Index, *TST* Total Sleep Time, *WASO* Wake After Sleep Onset, *SE* Sleep Efficiency

* $p < 0.05$

Table 3 Baseline comparison between HCs and ID patients of rsFC within EN

Comparison	ROI	ROI	p value (FDR)	t value
ID patients > HCs	ACC_R	DLPFC_L	0.044	2.045
	Thalamus_R	Amygdala_L	0.005	2.907
	Thalamus_L	Amygdala_L	0.002	3.170
	Hippocampus_L	Thalamus_R	0.038	2.111
	Hippocampus_L	Thalamus_L	0.017	2.439
	DLPFC_L	Thalamus_R	0.027	2.247

Using two-sample t-test, cluster-level $p < 0.05$ (FDR corrected) represents statistical significance

ROI Region of interest, *ID* Insomnia disorder, *HCs* Healthy controls, *ACC* Anterior cingulate cortex, *DLPFC* Dorsolateral prefrontal cortex, *L* Left, *R* Right

Shown in Table 5; Fig. 6. However, there was no significant difference between before and after treatment in the sham acupuncture group (Fig. 7).

Correlation analysis results

We conducted a Pearson's correlation analysis between clinical outcome changes (post-treatment minus pre-treatment) and Fisher z-scores of rsFC changes (post-treatment minus pre-treatment) across all ID patients and within-group, with age, sex, education, and duration of insomnia as covariates. Correlation analysis showed that the increased rsFC between the right amygdala and left hippocampus was positively

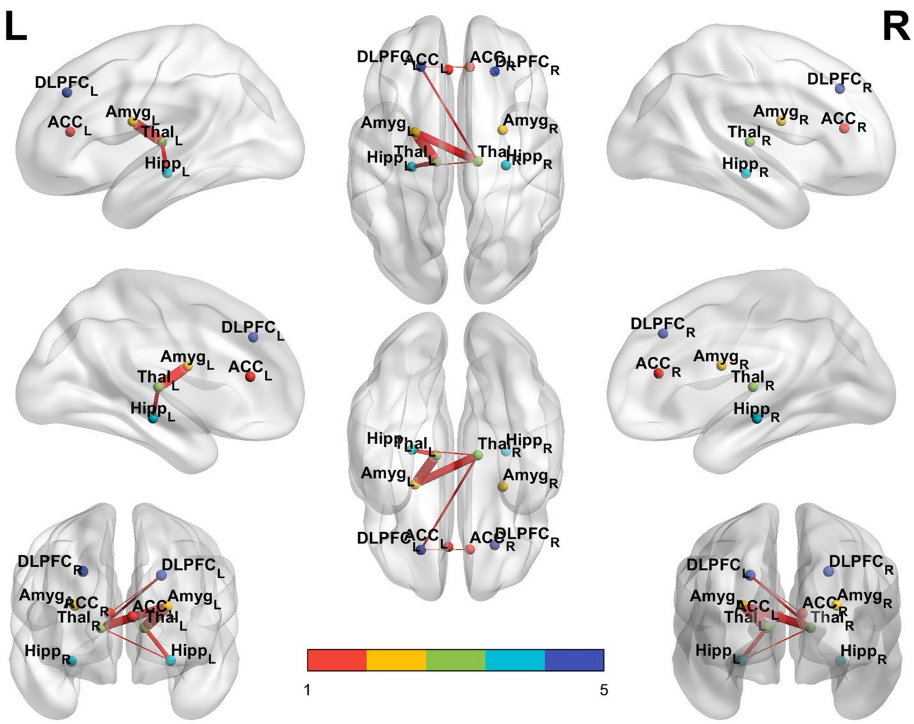

Fig. 4 Increased rsFC within the EN in ID patients compared with HCs in the baseline period. Five colors represent five brain regions (bilateral). Red lines indicate enhanced rsFC. The thickness of the line was positively correlated with the degree of increase. ACC, anterior cingulate cortex; DLPFC, dorsolateral prefrontal cortex; Amyg, amygdala; Thal, thalamus; Hipp, hippocampus; L, left; R, right

Table 4 Inter-group comparison of rsFC within EN regions

Comparison	ROI	ROI	*p* value (FDR)	t value
Real > Sham	ACC_R	ACC_L	0.006	2.881
	Hippocampus_L	ACC_R	0.044	2.066
	Amygdala_R	ACC_L	0.027	2.276
	Hippocampus_L	ACC_L	0.008	2.779
	Amygdala_L	Amygdala_R	0.020	2.400
	Hippocampus_L	Amygdala_R	0.014	2.552

Using two-sample t-test, cluster-level $p < 0.05$ (FDR corrected) represents statistical significance

ROI Region of interest, *Sham* Sham acupuncture group, *Real* Real acupuncture group, *ACC* Anterior cingulate cortex, *L* Left, *R* Right

correlated with the decreased HAMA scores ($r = 0.309$, $p_{uncorrected} = 0.035$, $p_{Bonferroni-corrected} = 0.245$) across all ID patients (Fig. 8). The decreased left amygdala rsFC with the left thalamus was negatively correlated with sleep efficiency (r = -0.514, $p_{uncorrected} = 0.012$, $p_{Bonferroni-corrected} = 0.084$) in real acupuncture group (Fig. 9). These two correlation results did not survive after Bonferroni correction, but showed a significant trend. No correlation was found between other significant Δ rsFC (post - pre) and Δ clinical outcomes (post - pre) (all $p_{uncorrected} > 0.05$).

Discussion

We investigated the rsFC changes within EN of insomnia patients evoked by real and sham acupuncture. In previous fMRI studies, the method of seed-based FC analysis had been proved reliable for analyzing the functional changes within networks [55, 56]. Ten brain regions (ROIs) within the emotional network were selected to calculate the rsFC values between each ROIs [55]. In this study, we observed abnormalities in rsFC centered on the thalamus and DLPFC within EN of ID patients compared to HCs. Moreover, compared to the sham acupuncture group, the real acupuncture group significantly increased rsFC of the ACC, hippocampus, and amygdala after 4-weeks treatment. Intra-group comparisons revealed that in the real acupuncture group, the rsFC between the left amygdala and left thalamus decreased after treatment compared with baseline. Although no statistically significant correlation was found after multiple comparisons, a trend of correlation was shown that the increased rsFC value between the right amygdala and left hippocampus was positively correlated with the decreased HAMA scores across all ID patients, and the decreased left amygdala rsFC value with the left thalamus was negatively correlated with sleep efficiency in real acupuncture group. These findings suggest that real acupuncture shows a positive effect on reducing anxiety and improving sleep

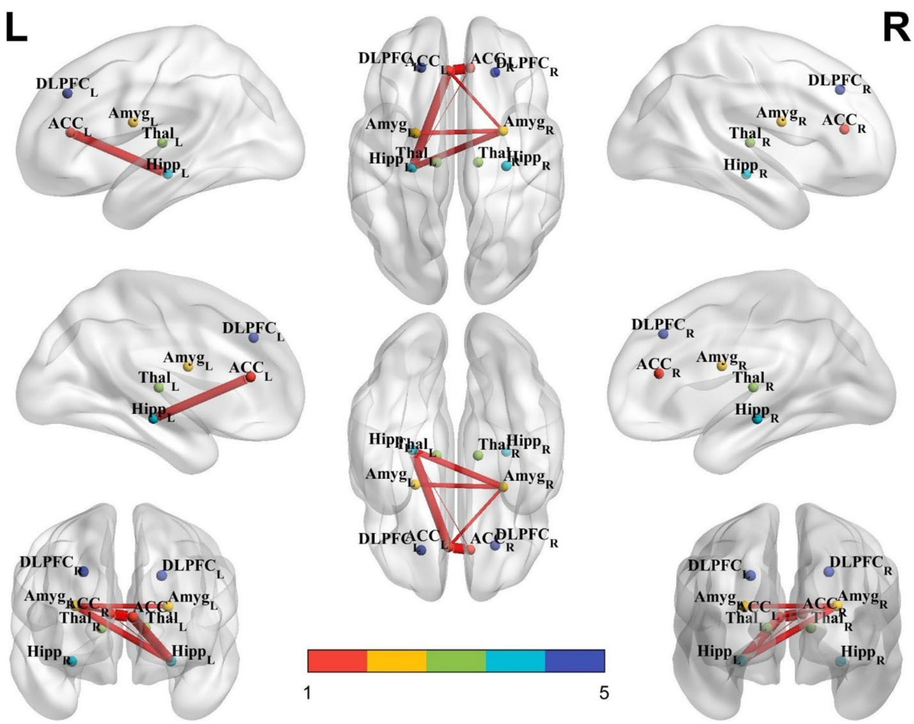

Fig. 5 Increased rsFC within the EN in real acupuncture group compared with sham acupuncture group after 4 weeks of acupuncture treatment. Five colors represent five brain regions (bilateral). Red lines indicate enhanced rsFC. The thickness of the line was positively correlated with the degree of increase. ACC, anterior cingulate cortex; DLPFC, dorsolateral prefrontal cortex; Amyg, amygdala; Thal, thalamus; Hipp, hippocampus; L, left; R, right

Table 5 Intra-group comparison of rsFC within EN (Real acupuncture group)

Comparison	ROI	ROI	*p* value (FDR)	t value
Post < Pre	Amygdala_L	Thalamus_L	0.009	-2.719

Using paired t-test, cluster-level $p < 0.05$ (FDR corrected) represents statistical significance

ROI Region of interest, *Post* Post-treatment, *Pre* Pre-treatment, *L* Left, *R* Right

efficiency by modulating the connectivity within the emotional related area.

The hippocampus, as one of the principal hubs of the limbic system and EN [61], is important for emotion regulation [62]. Studies have shown that hippocampal volume reduction is associated with difficulty maintaining sleep, hyperarousal, and cognitive dysfunction [63, 64]. Moreover, changes in neurotransmitters such as 5-HT and nitric oxide in the hippocampus [65] interfere with normal sleep-wake patterns and result in insomnia. ACC is another key brain region of the limbic system and EN [61]. Dysregulation of the dorsal ACC is associated with depression and obsessive-compulsive disorder [66]. Plante et al. found abnormal functional activity of the ACC in patients with major depression and ID [67]. A study showed that the functional connection between the hippocampus and ACC is associated with recollect episodic memory [68]. The circuit of ACC to the hippocampus controls the expression of contextual fear generalization, while manipulating the circuit may prevent anxiety and fear [69]. In addition, Feng et al. found that pharmacological and physical interventions can increase rsFC of hippocampus-ACC, which is negatively associated with PSQI score [70]. Moreover, some studies found that acupuncture could increase the FC strength of the ACC in patients with depression [71] and the FC of the hippocampus in older adults with cognitive decline [72]. In our trial, we found real acupuncture could increase rsFC of the bilateral ACC-left hippocampus compared with sham acupuncture. Thus, increasing the activity of hippocampus and its FC with ACC may have a positive effect on emotion regulation [73]. We suggest that the enhanced rsFC between ACC and hippocampus may be an intrinsic mechanism for decreased negative emotion in ID patients.

The amygdala plays a critical role in the formation and storage of memory associated with mood disorders, fear, and pain [74]. Gong et al. reported that amygdala is engaged in controlling emotion and has strong bidirectional interaction with the prefrontal cortex and ACC [75].

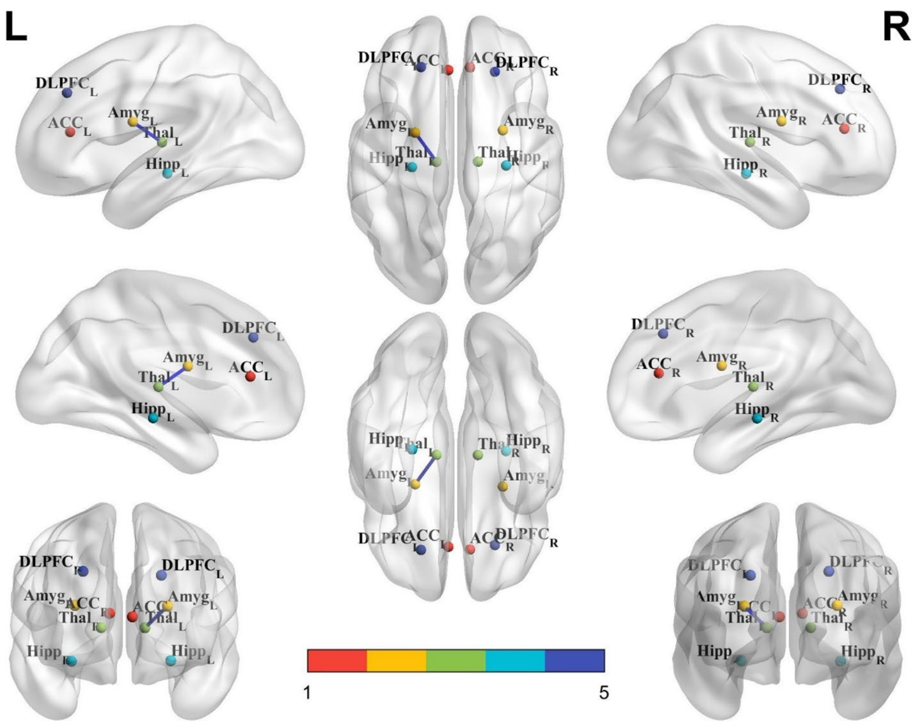

Fig. 6 Decreased rsFC within the EN after 4 weeks of treatment compared with baseline in the real acupuncture group. Five colors represent five brain regions (bilateral). Blue lines indicate decreased rsFC. The thickness of the line was positively correlated with the degree of decline. ACC, anterior cingulate cortex; DLPFC, dorsolateral prefrontal cortex; Amyg, amygdala; Thal, thalamus; Hipp, hippocampus; L, left; R, right

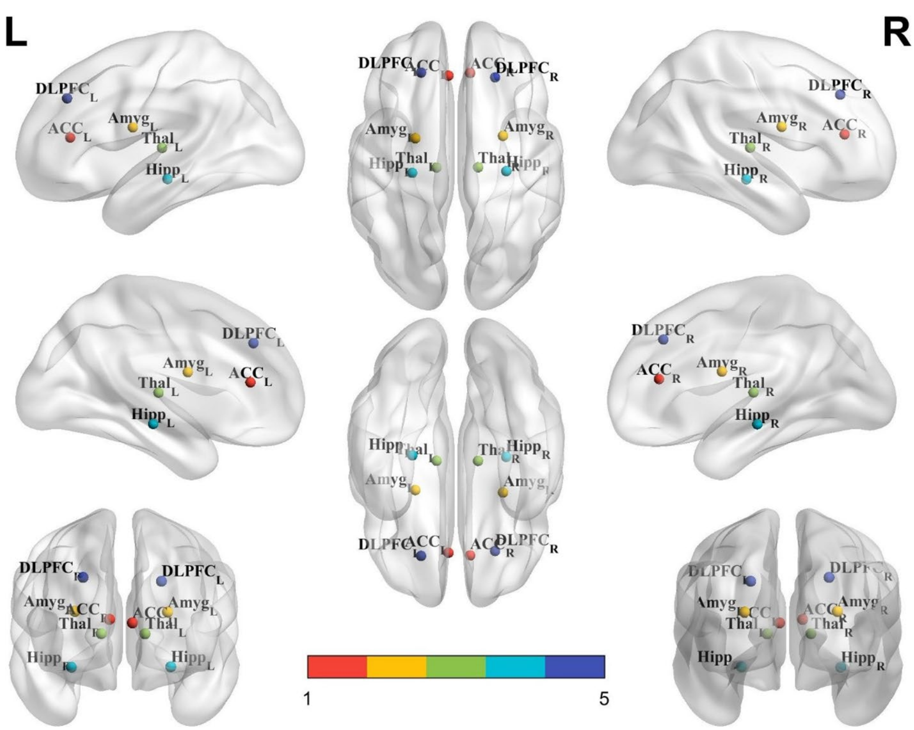

Fig. 7 There is no significant difference between before and after treatment in the sham acupuncture group. Five colors represent five brain regions (bilateral). ACC, anterior cingulate cortex; DLPFC, dorsolateral prefrontal cortex; Amyg, amygdala; Thal, thalamus; Hipp, hippocampus; L, left; R, right

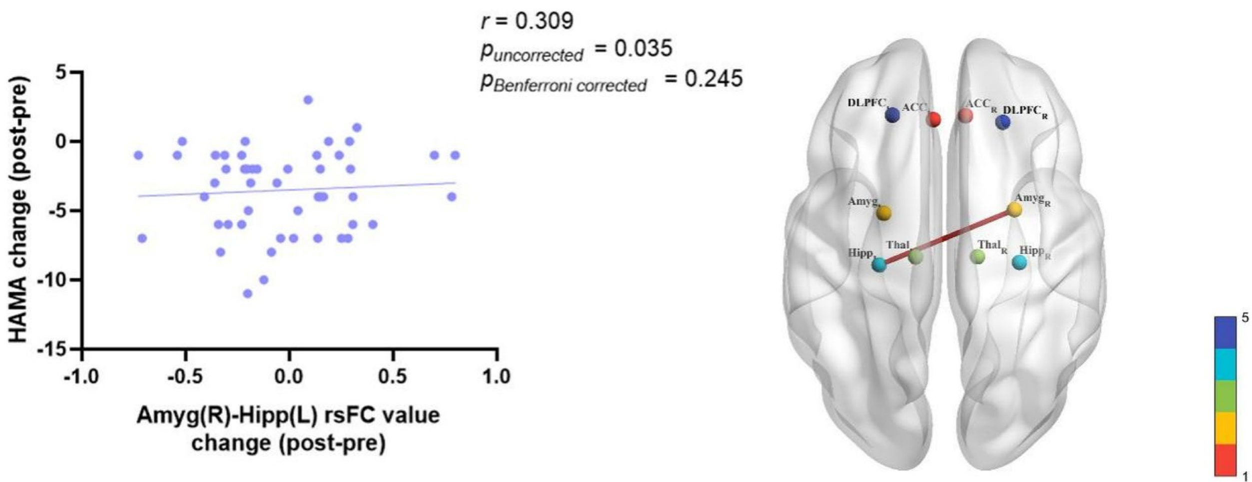

Fig. 8 The positive correlation tendence between rsFC value change in right amygdala with left hippocampus and HAMA change across all ID patients. Five colors represent five brain regions (bilateral). HAMA, Hamilton Anxiety Scale; ACC, anterior cingulate cortex; DLPFC, dorsolateral prefrontal cortex; Amyg, amygdala; Thal, thalamus; Hipp, hippocampus; L, left; R, right

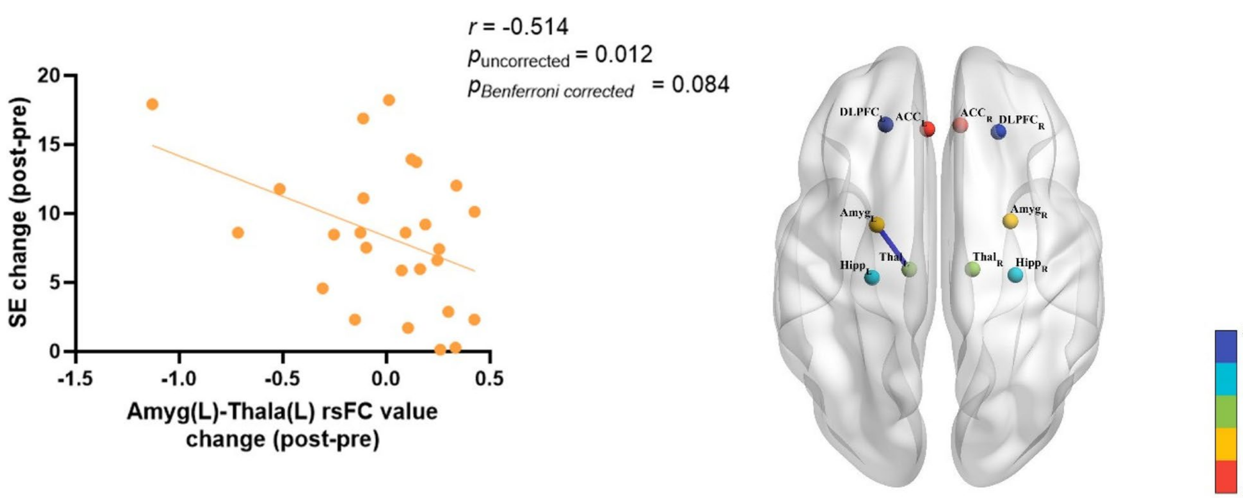

Fig. 9 The negative correlation tendence between rsFC value change in left amygdala with left thalamus and SE change in real acupuncture group. Five colors represent five brain regions (bilateral). SE, sleep efficiency; ACC, anterior cingulate cortex; DLPFC, dorsolateral prefrontal cortex; Amyg, amygdala; Thal, thalamus; Hipp, hippocampus; L, left; R, right

Therefore, abnormal amygdala activity has been involved in the neuropathology of major depression and anxiety disorder [76, 77], as well as insomnia [78]. In previous studies, the amygdala showed abnormal rsFC with the hippocampus [79, 80] in both insomnia and depression patients. Li et al. confirmed that neurofeedback training could alter amygdala activity, which reshaped the abnormal FC caused by insomnia and improved the sleep of ID patients [30]. Furthermore, a study found that acupuncture significantly modulated FC between the amygdala and hippocampus which was related to decreased anxiety and depression [81]. Wang et al. found that acupuncture treatment significantly increased rsFC between the amygdala, ACC, and parahippocampus, and the rsFC strength between the amygdala and ACC was positively associated with the depression level [31]. In this study, we also found after real acupuncture treatment, rsFC between bilateral amygdala, right amygdala rsFC with left ACC and left hippocampus were increased. In addition, there was a positive correlation trend between the increased rsFC of the right amygdala and left hippocampus and decreased scores of HAMA. Thus, we speculate that acupuncture may decrease anxiety mood by modulating the connectivity between amygdala and hippocampus.

The thalamus has a crucial role in the integration of sensory, emotional, and cognitive information [82, 83]. Recent studies suggested that the negative mood of insomnia patients was associated with dysregulation of thalamus [84, 85]. Kim et al. indicated that increased thalamocortical activity was related to hypersensitive sensory during sleep time [86]. Kong et al. found that acupuncture altered the rsFC between the thalamus and DMN regions, which may be the mechanism by which acupuncture regulates sensory and emotional changes caused by chronic pain [87]. Zhao et al. indicated that transcutaneous auricular vagus nerve stimulation modulates thalamus-related FC in ID patients, suggesting a potential target for insomnia treatment [88]. In this study, we found increased rsFC in the thalamus-amygdala and thalamus-hippocampus in ID patients compared with HCs, which may be an intrinsic mechanism for the abnormal emotional sensitivity of ID patients. Further, our results revealed that real acupuncture could decrease the rsFC between the left thalamus and left amygdala, and the rsFC showed a negative trend with sleep efficiency. The ACC, amygdala, and thalamus all belong to the autonomic nuclei of the brain and are affected by inputs from the internal and external environments [89]. Acupuncture as an effective stimulus could modulate the autonomic nervous system and further regulate the ACC, amygdala, and thalamus to relieve insomnia and negative moods. Therefore, this may be the neuro-mechanism of acupuncture in improving sleep in ID patients.

DLPFC is a major prefrontal region involved in high-level cognitive and complex emotion processing [90]. Many studies have found that increased DLPFC activity was associated with adverse emotions [91] and excessive self-attention [92]. Consistent with previous studies, our results showed increased rsFC in the DLPFC-ACC and DLPFC-thalamus in ID patients. However, no differences in rsFC of DLPFC were found in inter-group and intra-group comparisons after acupuncture treatment. We speculate that the DLPFC contains a large number of voxels and has different intracerebral regional divisions [93]. Further analysis could be conducted from more subdivided regions.

Acupuncture has been proven to be effective in decreasing hyperarousal level and regulating emotions of ID sufferers [94]. Meanwhile, it could modulate heart rate variability (HRV), indicating that acupuncture can help restore the balance between the sympathetic and parasympathetic nervous system [95]. It has been shown that regions associated with autonomic regulation are mainly ACC, prefrontal cortex, and amygdala [96], which are also responsible for emotional management. The mechanism of modulation of the emotional network by acupuncture may be related to the regulation of sympathetic and parasympathetic nerves balance and the further alteration of FC in EN. Future research should combine HRV and fMRI to explore the central mechanism of acupuncture in regulating sleep and emotions of insomnia sufferers.

There are several limitations to our study. First, the intervention in the sham acupuncture group inevitably has some nonspecific physiological effects. As a type of physical placebo, sham acupuncture necessarily involves touching, which may cause other types of nonspecific physiological effects to occur in addition to the placebo response [97]. Studies have shown that the amygdala, ACC, and PFC are also associated with the processing of painful stimulus signals [98, 99]. A perfect comparison of real acupuncture should be developed in the future, such as designing a sham acupuncture with blunt tip needle so it will not penetrate skin. Second, no sleep poly-conductivity test was carried out in this study, so there was a lack of more accurate judgment on the quality, quantity, and structure of sleep. Polysomnography (PSG) provides a quantitative assessment of nocturnal sleep and is an important tool in the diagnosis of ID [100, 101]. Hence, PSG could be combined with fMRI technology to explore the central nervous mechanisms of acupuncture for insomnia in future studies. Third, there is no statistically significant correlation found after Bonferroni correction; we speculate that it may be related to the sample size limitations. Fourth, the blinding of participants was not assessed in this study. Thus, the blinding questionnaire should be collected in future acupuncture studies. Fifth, due to the specificity of fMRI studies, there are no cut-offs of normality in relation to fMRI results that can be used as comparative parameters. Thus, we compared the results with the literature previously to elucidate the relationship between acupuncture and rsFC changes within EN. Future studies should further explore the more definitive and consistent neuroimaging mechanisms of acupuncture for ID. Sixth, because insomnia is also closely related to the default mode network, salience network [78], and cognitive control network [102], the synergy between brain networks can be analyzed from the perspective of large-scale brain networks in the future. Last, subgroup analyses of insomnia were not performed. Future research should expand the sample size and classify ID into different subtypes, such as difficulty falling asleep and early-awakened insomnia, to explore the neuroimaging differences between different insomnia subtypes.

Conclusion

Our results demonstrated that there is a general increase of rsFC within the EN in ID patients, as well as that real acupuncture could modulate the rsFC of the ACC, hippocampus, and amygdala. Moreover, increased rsFC value in the right amygdala-left hippocampus and decreased rsFC value in the left amygdala-left thalamus were correlated with reduced anxiety and improved sleep efficiency. The findings support the hypothesis that acupuncture may improve sleep and emotion by modulating functional activity of emotion-related regions in ID patients. The results highlight the potential mechanism in acupuncture treatment of insomnia.

Abbreviations

ACC	Anterior cingulate cortex
CSF	Cerebrospinal fluid
DLPFC	Dorsolateral prefrontal cortex
EN	Emotional network
FA	Flip angle
FC	Functional connectivity
FDR	False discovery rate
FOV	Field of view
HAMA	Hamilton Anxiety Scale
HAMD	Hamilton Depression Scale
HAS	Hyperarousal Scale
ID	Insomnia disorder
ITT	Intention-to-treat
MNI	Montreal Neurological Institute
PFC	Prefrontal cortex
PSQI	Pittsburgh Sleep Quality Index
ROI	Regions of interest
SE	Sleep efficiency
SN	Salience network
TST	Total sleep time
WASO	Wake after sleep onset

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12906-024-04612-0.

Supplementary Material 1.

Supplementary Material 2.

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Authors' contributions
Jing Guo: conception and study design. Xue-Jiao Yin: data collection or acquisition. Zhao-Yi Chen: statistical analysis. Jiao Liu and Zhong-Jian Tan: fMRI data reviewing and analysis. Bin Li: review and editing, methodology. Gui-Ling Wang: clinical intervention design. Tong-Fei Jiang. and Jing Guo: drafting the manuscript work. Jing Guo: revising the manuscript critically for important intellectual content. All authors contributed to the article and approved the submitted version.All authors reviewed the manuscript.

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Availability of data and materials
The raw data supporting the conclusions of this article can be obtained on reasonable request from the corresponding author. Requests to access the datasets should be directed to JG, guojing_2002@163.com.

Declarations

Ethics approval and consent to participate
This trial was performed at the Beijing Hospital of Traditional Chinese Medicine, Capital Medical University (Beijing TCM Hospital). It was approved by the ethics committee of the hospital and registered on http://www.chictr.org.cn (Chinese Clinical Trials Register, ChiCTR1800015282). Informed consent was obtained from all subjects in this study. A copy of the informed consent is available for review by the Editor-in-Chief of this journal.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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