Non-pharmacological and pharmacological interventions relieve insomnia symptoms by modulating a shared network: A controlled longitudinal study

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ABSTRACT

Background: Primary insomnia (PI) is one of the most common complaints among the general population. Both non-pharmacological and pharmacological therapies have proven effective in treating primary insomnia. However, the underlying mechanism of treatment remains unclear, and no studies have compared the underlying mechanisms of different treatments.

Methods: In this study, we investigated gray matter volume (GMV) and resting-state functional connectivity (rsFC) changes following both pharmacological and non-pharmacological treatments in patients with PI. A total of 67 PI patients were randomized into benzodiazepine treatment, cupping treatment, or a wait-list control group for 4 weeks. The Pittsburgh Sleep Quality Index (PSQI), gray matter volume (GMV), and resting-state functional connectivity (rsFC) of the hippocampus were measured at the beginning and end of the experiment.

Results: We found 1) significantly decreased PSQI scores in the cupping and benzodiazepine treatment groups compared to the control group with no significant differences between the two treatment groups; 2) significant GMV increases in the cupping group compared to the control group at the right hippocampus after 4 weeks of treatment; 3) significantly increased rsFC between the right hippocampus and left rostral anterior cingulate cortex/medial prefrontal cortex (rACC/mPFC) in the two treatment groups, which was significantly associated with PSQI score decreases.

Discussion: Our findings suggest that benzodiazepine and cupping may share a common mechanism to relieve the symptoms of patients with PI.

1. Introduction

Primary insomnia (PI) is one of the most prevalent chronic sleep disorders and affects approximately 2–4% of the general population (Ohayon, 2002). Long-standing primary insomnia can reduce quality of life, hinder work productivity, impair social function, and become life-threatening (Riemann and Voderholzer, 2003; Shekleton et al., 2010). As a result, insomnia has become the second major cause of patient visits to neurological clinics, after headaches (Wade, 2010).

Recent brain imaging studies have indicated that insomnia is associated with anatomical and functional alterations in neural systems involved in general arousal, emotion and reward, and prefrontal cognitive control (Khazaie et al., 2017; Spiegelhalder et al., 2015). Literature suggests that brain regions such as the hippocampus (Joo et al., 2014), rostral anterior cingulate cortex (rACC) (Winkelman et al., 2013), orbitofrontal cortex (OFC) (Altena et al., 2010), dorsolateral prefrontal cortex (DLPFC) (Huang et al., 2017), and amygdala (Baglioni et al., 2014) are involved in the pathophysiology and development of insomnia.

One of the most well-studied regions in insomnia studies is the

hippocampus (Neylan et al., 2010; Riemann et al., 2007; Taki et al., 2012). As a subcortical structure, the hippocampus is crucially involved in learning and memory formation, encoding of spatial information, and emotion regulation (Girardeau et al., 2017; Kreutzmann et al., 2015). Animal studies indicate that prolonged stress exposure and sleep loss can suppress hippocampal cell proliferation and neurogenesis (Bhagya et al., 2017; Murata et al., 2017), which in turn affects local structural and functional integrity, as well as the interaction of the hippocampus (Bhagya et al., 2017). In previous studies, findings on abnormal hippocampal structure in insomnia are mixed. Some studies have reported a reduced bilateral hippocampus volume in insomnia patients (Joo et al., 2014; Riemann et al., 2007) or a negative association between hippocampal atrophy and cognitive impairment (Koo et al., 2017). In contrast, other studies have found that the hippocampus volumes in individuals with PI did not significantly differ from those of normal sleepers (Noh et al., 2012; Spiegelhalder et al., 2013; Winkelman et al., 2010). These contrasting results could be due to differences in anatomical delineation of the hippocampus and differences in insomnia duration (O'Byrne et al., 2014).

In a recent study, Leerssen et al. (Leerssen et al., 2018) found that relative to healthy controls, patients with insomnia disorder showed a significantly stronger connectivity of the bilateral hippocampus with the left middle frontal gyrus. The individual differences in the strength of this connectivity were associated with insomnia severity and subjective sleep quality. In another recent study (Lee et al., 2018), investigators found that PI patients exhibited weaker FC between the left hippocampus and left fusiform gyrus compared to controls, and these altered brain responses reversed after five sessions of cognitive-behavioral therapy. Taken together, these studies demonstrate the important role of the hippocampus in the neuropathology of insomnia.

Pharmacological intervention play an important role in the treatment of insomnia (Holbrook et al., 2001; Winkler et al., 2014). However, it is associated with unwanted side effects (Holbrook et al., 2001). The cognitive behavioral therapy for insomnia (CBT−I) is the mainstay of non-pharmacologic management of chronic insomnia (Morin, 2006; Trauer et al., 2013). Problems with accessibility and cost effectiveness mean that many chronic insomniacs do not benefit from this treatment (Kaystacey and Attarian, 2016).

Recently, other non-pharmacological methods, such as aerobic physical exercise, music therapy, acupuncture, and cupping (Altena et al., 2008; Kim et al., 2017; Lee et al., 2018; Stoffers et al., 2013; Wilt et al., 2016; Yeung et al., 2012a,b; Yeung et al., 2011) have drawn the attention of investigators. Cupping therapy, a promising and safe non-pharmacological treatment originating from China, is applied by placing cups on selected locations to create suction and produce hyperemia or hemostasis. Some researchers believe that cupping may suppress the proliferation of harmful inflammatory mediators, biological, chemical, or other unwanted substances, and increase the flow of blood to the skin and muscle, stimulating peripheral nerves, neurohormones, and the circulatory and immune systems (El Sayed et al., 2013; Niasari et al., 2007). In a previous study (Wang et al., 2015a, 2015b), we found that cupping can significantly improve the quality of sleep and alleviate anxiety and depression symptoms in insomniacs compared with pharmacologic treatments. The beneficial effect of cupping to relieve the symptoms of insomnia have also been achieved from other studies (Li and Zhang, 2013; LI et al., 2013; Zhang et al., 2010; Zhu et al., 2013). Nevertheless, the underlying mechanism of cupping remains unknown.

The present study aims to comparatively investigate the treatment effects of benzodiazepines and cupping, as well as their underlying mechanisms, by investigating the brain structural and functional connectivity changes after different treatments in patients with PI. Based on previous findings (Altena et al., 2010; Joo et al., 2014; Neylan et al., 2010; Riemann et al., 2007), we hypothesized that hippocampal volumetric alterations would be reversible upon successful therapy, and that both non-pharmacologic and pharmacologic interventions would modulate similar hippocampus-related rsFC changes in patients with PI.

2. Methods

2.1. Subjects

Subjects with primary insomnia were recruited. The experiment was approved by the Institutional Review Board of the Affiliated Hospital of Chengdu University of Traditional Chinese Medicine (CDUTCM) and was registered at www.chictr.org.cn (No. ChiCTR-ICR-15006021). All subjects signed a written consent form and agreed to allow their data to be analyzed.

Inclusion criteria were: (1) between 18 and 65 years of age; (2) an independent psychiatric syndrome (primary insomnia) as defined by the fourth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) and diagnosed by study physician; (3) at least three months of difficulty falling asleep, maintaining sleep, or early awakening; and (4) Willing to stop taking medication or receiving any other treatment four weeks before beginning the intervention. Exclusion criteria were: (1) clinical evidence of any moderate to severe sleep disorder other than insomnia, such as hypersomnia, parasomnia, or sleep-related movement disorder; (2) insomnia caused by serious organic disease or severe mental disease secondary to depression (Self-Rating Depression Scale > 70) (Lennard et al., 2011) or generalized anxiety (Self-Rating Anxiety Scale > 70); (3) abnormal findings, such as infarction or focal lesion, on conventional brain MR images; (4) pregnant, nursing, or lactating; and (5) MRI contraindications, such as claustrophobia, metallic implants, or devices in the body.

2.2. Experimental procedure

This study was a pragmatic randomized, open-labeled neuroimaging trial with three arms. After screening, eligible participants were randomized into one of three groups: (i) cupping group; (ii) benzodiazepine group; (iii) wait-list control group (Fig. 1). Patients were instructed to complete a diary booklet each day to describe any side effects they believed to be related to treatment.

2.3. Cupping treatment

All cupping was applied by licensed Traditional Chinese Medicine doctors with over 5 years of experience in the clinical practice. Acupoints applied in the cupping group were identical to our previous randomized clinical trial (Wang et al., 2015a, 2015b). Details of the cupping technique are shown in Supplementary Table 1 and Fig. 2. The entire treatment totaled 20 min. Participants in the cupping group received cupping treatment twice a week for four weeks, totaling up to eight treatment sessions.

2.4. Benzodiazepine treatment

Patients in the benzodiazepine group were instructed to take Estazolam tablets (2nd branch of Sinepharm, Shanghai Pharmaceutical. Ltd. Patch No. 060301–01, Strength 1 mg/tablet) daily for four weeks. Estazolam (1 mg) was given 30–60 min prior to bedtime every day. A 2 mg dosage of Estazolam was allowed for patients if the Pittsburgh Sleep Quality Index (PSQI) was greater than or equal to 14.

2.5. Wait-list control

Patients in the control group did not receive any treatment. After the completion of the full observational period, patients in the control group were provided compensation for transportation, and free cupping treatment was provided if required.

2.6. Clinical outcomes and data analysis

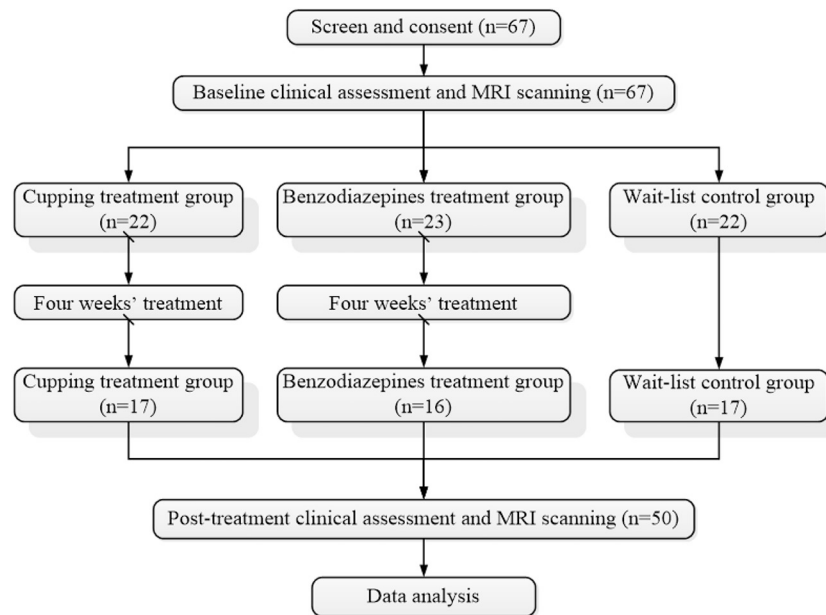All clinical outcomes were measured at week 0 (baseline) and week

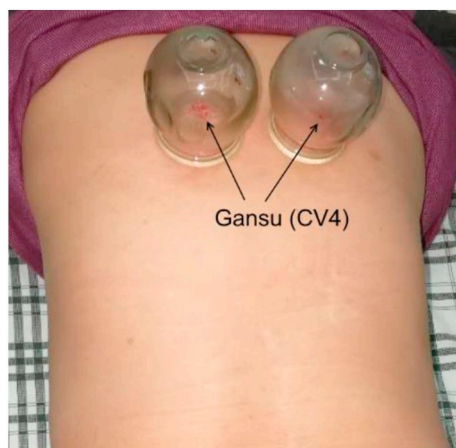Fig. 1. Study flow chart. MRI: magnetic resonance imaging.

Fig. 2. Illustration of the cupping therapy for primary insomnia.

4 (post-treatment). The primary measurement was the Pittsburgh Sleep Quality Index (PSQI). In addition, the Self-Rating Anxiety Scale (SAS) and Self-Rating Depression Scale (SDS) were applied as secondary clinical outcomes.

Clinical outcome analysis was performed using SPSS 22.0 Software (SPSS Inc., Chicago, IL, USA). One-way ANOVA and Chi-square tests were applied to compare the baseline characteristics of the subjects among groups. An ANCOVA was applied to compare the changes in PSQI, SAS, and SDS scores across the three groups, with age, gender, and duration as covariates. Post-hoc analysis with Bonferroni correction was applied to explore between-group differences.

2.7. Imaging acquisition

MRI scans were performed on a 3.0-Tesla magnetic resonance scanner (GE Healthcare Discovery MR750) in the Department of Radiology at the Affiliated Hospital of CDUTCM. A high-resolution 3D T1-weighted brain volume (BRAVO) MRI sequence was applied with the following parameters: repetition time (TR) = 8.16 ms, echo time (TE) = 3.18 ms, flip angle = 7°, field of view (FOV) = $256 \times 256\,\text{mm}^2$, in-plane resolution = $1 \times 1 \times 1\,\text{mm}^3$, and 188 slices. Resting-state fMRI were obtained with a gradient-echo T2*-weighted echo-planar

imaging sequence (GRE-EPI): time point = 201, TR/TE = 2000/30 ms, matrix = $64 \times 64 \times 30$, voxel size = $3.5 \times 3.5 \times 4.02\,\text{mm}^3$, flip angle = 90°). During the MRI, all patients were instructed to relax with their eyes closed without falling asleep.

2.8. Brain structure analysis using VBM

VBM was performed using Statistical Parametric Mapping (SPM12) (Wellcome Department of Cognitive Neurology, University College, London, UK) running under a MATLAB suite (MathWorks, Inc., Natick, Massachusetts). First, all images were checked for artifacts, structural abnormalities, and pathologies. Then, the images were segmented into gray matter (GM), white matter, and cerebrospinal fluid (CSF) and normalized using the high dimensional DARTEL algorithm (Ashburner, 2007). Subsequently, a group specific template was created to reduce variability between participants. The template was then used to normalize the images into the standard Montreal Neurological Institute (MNI) space using the "DARTEL Normalize to MNI Space" program and the "preserve amount" option to retain the volumetric data of the original images. Finally, spatial smoothing was performed with an isotropic Gaussian kernel of 8 mm full-width at half maximum.

Group analysis was applied using a random effects model. We first compared the pretreatment regional gray matter volume (GMV) differences between the three groups using a one-way ANOVA. Then we performed second level analyses using a factorial design module in SPM12 to explore the differences among the three groups. There were two factors included in the analysis. The first factor had three levels (cupping group, benzodiazepine group, and control group), and the second factor had two levels (pre-treatment and post-treatment). Age (year), gender, and duration (month) were also included in the model as covariates. Similar to a previous study (Kalmady et al., 2014), an absolute threshold of 0.1 was used for masking. Total intracranial volume was obtained by summing up the overall volumes of GM, white matter, and CSF.

Given the important role of the hippocampus in neural plasticity changes following insomnia (Joo et al., 2014; Khazaie et al., 2017), the left and right hippocampus, derived from automated anatomical labeling (AAL) using WFU-Pick Atlas software (Maldjian et al., 2003), were selected as regions of interest (ROIs). For between-group comparisons, we applied a threshold of $p < .001$ and small volume false discovery rate (FDR) corrected $p < .05$ in the ROI as defined above. A

threshold of voxel-wise < 0.001 uncorrected and cluster-level p < .05 FDR correction was applied to non-ROI brain regions.

2.9. Seed based functional connectivity analysis

Data preprocessing and calculations of functional connectivity were all preprocessed using the CONN toolbox version 17.f (http://www.nitrc.org/projects/conn) in MATLAB (Whitfield-Gabrieli and Nieto-Castanon, 2012).

The left and right hippocampus were used as seeds. Preprocessing was performed with a default pipeline. The specific steps are as follows: slice timing correction; head motion correction; skull-stripping using BET; co-registration of the anatomical image to the mean functional image; segmentation of the anatomical gray matter, white matter, and CSF; normalization to MNI152 standard template; and smoothing with an 8-mm Gaussian kernel (Whitfield-Gabrieli and Nieto-Castanon, 2012). Band-pass filtering was performed with a frequency window of 0.01–0.08 Hz.

First-level correlation maps were produced by extracting the BOLD time course from each hippocampal seed and by computing Pearson's correlation coefficients between that time course and the time courses of all other voxels in the brain. Correlation coefficients were Fisher transformed into 'Z' scores to increase normality and allow for improved second-level General Linear Model analyses.

Whole-brain second level group analysis was applied using two sample t-tests to compare the hippocampal functional connectivity changes between different groups. Age, gender, and duration were included as covariates of non-interest. A threshold of voxel-wise $p < .005$ uncorrected and cluster-level p < .05 FDR corrected was applied in data analysis.

To explore the association between the rsFC changes and PSQI improvement, we also extracted the average z score values of the significantly altered rsFC clusters before and after the treatment (cupping versus control and benzodiazepines versus control), and performed partial correlation analysis using SPSS 22.0 Software to test the association between the significantly altered rsFC clusters and PSQI changes, controlling for age, gender, and duration.

3. Results

Seventy-three patients with PI were recruited for this study. Of the 67 participants who passed screening and finished baseline scans, 50 (17 in the cupping treatment group, 16 in the benzodiazepine treatment group, 17 in the wait-list control group) completed the study. Seventeen patients did not participate in the second fMRI scan due to scheduling conflicts (5 in the cupping group, 7 in the benzodiazepine group, 5 in the control group). A flowchart of this study can be found in Fig. 1. Of the 50 patients who participated in the two fMRI scans, 2 patients were excluded from data analysis due to incomplete scans (lack of resting state fMRI or T1 anatomy; 1 in the benzodiazepine group, 1 in the control group).

3.1. Clinical outcomes

Baseline characteristics for the 50 patients who completed the study are detailed in Table 1. There were no significant differences in age, gender, duration, PSQI score, SAS score, and SDS score among the three groups at baseline (all $p > .05$).

ANCOVA on PSQI score change (post-treatment minus pre-treatment) showed a significant group difference among the three groups ($F_{(2,42)} = 73.982$, $p < .001$). Post-hoc analysis showed that the cupping and benzodiazepine groups produced a significant decrease in PSQI scores compared to the control group (cupping vs control: $p_{Bonferroni} < 0.001$; Cohen's d = 2.07; benzodiazepines vs control: $p_{Bonferroni} < 0.001$, Cohen's d = 2.17). There was no significant difference among the two treatment groups in PSQI score change (Cohen's

Table 1
Demographics and clinical outcome measurements (mean ± SD).

Characteristic	Cupping (n = 17)	Benzodiazepines (n = 16)	Wait-list (n = 17)
Age (years)	36.53 ± 10.64	42.63 ± 10.63	39.76 ± 11.10
N (Female/Male)	17 (11/6)	16 (8/8)	17 (11/6)
Duration (months)	56.29 ± 57.91	46.19 ± 49.07	41.81 ± 48.59
Baseline			
PSQI	13.65 ± 1.69	14.56 ± 2.03	14.00 ± 2.32
SAS	55.18 ± 4.95	54.25 ± 5.50	53.06 ± 4.42
SDS	54.59 ± 8.02	52.19 ± 9.77	52.88 ± 6.49
Endpoint after 4 weeks of treatment			
PSQI	7.94 ± 2.97	9.63 ± 2.97	14.65 ± 2.15
SAS	41.53 ± 4.61	39.33 ± 4.61	53.81 ± 3.47
SDS	44.29 ± 8.07	49.93 ± 7.57	53.44 ± 6.07
Change between baseline and endpoint			
PSQI	−5.71 ± 2.64*	−4.94 ± 2.08*	0.65 ± 3.20
SAS	−13.65 ± 4.12*	−15.20 ± 6.96*	0.81 ± 3.19
SDS	−10.29 ± 6.75*	−2.47 ± 4.32	0.63 ± 3.30

Abbreviations: PSQI, Pittsburgh Sleep Quality Index; SAS, Self-Rating Anxiety Scale; SDS, Self-Rating Depression Scale.
* Indicates significant difference in change when compared to wait-list control group.

d = 0.32) (Table 1). For the control group, there was no significant change from pre-treatment to post-treatment PSQI scores ($T = -1.518$, $p = .38$).

ANCOVA on secondary clinical outcomes showed that the two treatment groups both had lower SAS scores than the control group at 4 weeks (Table 1): cupping group (41.53 ± 4.61) and benzodiazepines (39.33 ± 4.61) compared with the control group (53.81 ± 3.47). ANOVA $p < .001$ (F(2,42) = 48.054) between the three groups; $p_{Bonferroni} < 0.001$ (Cohen's d = 3.92) between the cupping and control groups $p_{Bonferroni} < 0.001$ (Cohen's d = 2.96) between benzodiazepines and control group; $p_{Bonferroni} = 0.81$ (Cohen's d = 0.27) between cupping and benzodiazepines group. In addition, the cupping group had lower SDS scores than the other groups at 4 weeks (Table 1): cupping group (44.29 ± 8.07) compared with benzodiazepines (49.93 ± 7.57) and the control group (53.44 ± 6.07). ANOVA p < .001 (F (2,42) = 22.419) between the three groups; Bonferroni analysis $p_{Bonferroni} < 0.001$ (Cohen's d = 2.01) between the cupping and control groups; $p_{Bonferroni} < 0.001$ (Cohen's d = 1.38) between cupping and benzodiazepines group; $P = .26$ (Cohen's d = 0.81) between benzodiazepines and control group.

3.2. VBM analysis results

To explore the baseline differences in GMV, we first applied a comparison between all groups using a one-way ANOVA. No significant differences were observed among the three groups at baseline.

After 4 weeks of treatment, we observed significant GMV increases in the cupping group compared to the control group at the right hippocampus (cluster size 41, MNI peak coordinates: 38; −29; −14, small-volume corrected at FDR $p = .039$). There were no other significant findings. No significant differences were observed in other between-group comparisons (cupping vs. benzodiazepine and benzodiazepine vs. control).

Within group comparison showed no pre- and post-treatment differences across all three groups at the threshold we set. When we applied a relatively less conservative threshold of voxel-wise p < .001 uncorrected with 20 continuous voxels, we found that compared to the pre-cupping, the GMV in the right hippocampus, left putamen/insula, and right superior frontal gyrus increased after cupping treatment.

Table 2
Left/right hippocampal rsFC change (post-pre) differences among the three groups.

Region of interest	Contrast	Brain region	k	MNI			Z value
				x	y	z	
Left hippocampus	Cupping > control	Left rACC/mPFC	477	−12	48	14	3.62
Right hippocampus	Cupping > control	Left rACC/mPFC	445	−8	36	14	3.84
	Benzodiazepines > control	Left rACC/mPFC	397	−14	44	14	4.51

Note: Age, gender, and duration were included as covariates. The statistical threshold was set at p < .005, and the family-wise error was corrected for multiple comparisons.
Abbreviations: rACC, rostral anterior cingulate cortex; MNI, Montreal Neurological Institute; mPFC, medial prefrontal cortex.

3.3. Functional connectivity results

To explore whether there was a significant difference in head movement between the three groups, we extracted six average head movement parameters with CONN software and performed a one-way ANOVA. No significant difference was found in average head movement among the three groups (F = 0.42, p = .73).

There was significantly increased connectivity between the right hippocampus and left rostral anterior cingulate cortex/medial prefrontal cortex (rACC/mPFC) in the cupping group compared to controls after longitudinal treatment (Table 2; Fig. 3B, shown in red). Benzodiazepine treatment also produced greater rsFC increases between the right hippocampus and left rACC/mPFC compared to the control group (Table 2; Fig. 3B, shown in green). In addition, the cupping group showed increased left hippocampus and left rACC/mPFC compared to the control group (Table 2). There were no right/left hippocampal rsFC differences in any brain region between the cupping and benzodiazepine groups at the threshold we set.

Within group comparisons showed that there was significantly increased ('post' minus 'pre') rsFC between the right hippocampus and right putamen/insula (cluster size 472; MNI peak coordinates: 30, 2, 2; FDR p = .015) after treatment in the cupping group. There were no other significant pre- and post-treatment rsFC differences in all three groups.

To explore the association between rsFC changes and corresponding clinical outcome changes, we investigated the association between right-hippocampus-related rsFC activity and PSQI score changes in the cupping and benzodiazepine groups separately. The partial correlation between PSQI score decreases (clinical improvement) and right hippocampus and rACC/mPFC rsFC changes (scan 2 – scan 1) was significant in the cupping group (r = −0.65, p = .01) (Fig. 3A). Similarly, PSQI score decreases were significantly negatively associated with right

hippocampal and rACC/mPFC rsFC change in the benzodiazepine group (r = −0.65, p = .01) (Fig. 3C).

Further exploratory association found no significant association between the SAS and rsFC between the right hippocampus and rACC/mPFC rsFC in both cupping (p = .82) and benzodiazepine (p = .14) groups. There was also no significant association between the SDS and right hippocampus - rACC/mPFC rsFC in these two treatment groups (cupping, p = .99; benzodiazepine, p = .63).

4. Discussion

In the present study, we examined the effects of 4 weeks of pharmacological treatment (benzodiazepines) and cupping on primary insomnia, as well as their underlying mechanisms. We found that compared to the wait-list control group, both cupping and benzodiazepine treatment groups showed significant clinical remission of insomnia symptoms. VBM analyses revealed significant GMV increases at the right hippocampus after 4 weeks of cupping treatment compared to the control group. Seed-based rsFC analyses showed that both cupping and benzodiazepine treatments significantly increased rsFC between the right hippocampus and left rACC/mPFC compared to the control group. There was a negative association between insomnia symptom reduction and rsFC increases between the right hippocampus and left rACC/mPFC in the two treatment groups, implying that cupping and benzodiazepines may achieve treatment effects by modulating a common network.

Previous studies suggest that cupping can produce significant physiological effects. For instance, literature suggests that cupping can promote peripheral blood circulation (Ke et al., 2016), improve local anaerobic metabolism (Emerich et al., 2014), reduce inflammation (Lin et al., 2014), and modulate the cellular immune system (Seçilmiş, 2013). In this study, we found that compared to the no-treatment control group, both cupping and benzodiazepine treatment groups
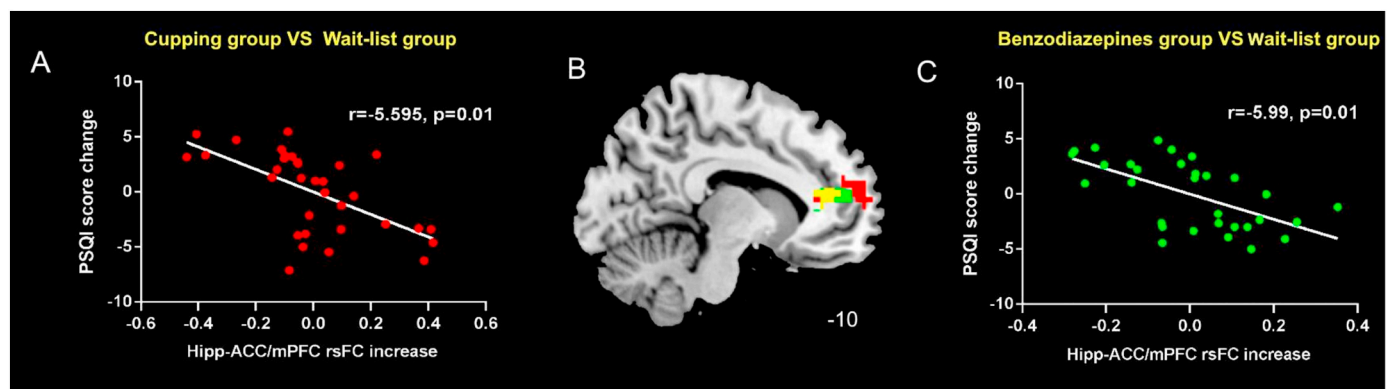
Fig. 3. (A) Shown in *red*: Greater increase in connectivity between the right hippocampus and the ACC/mPFC in the cupping group than in the control group. Shown in *green*: Greater increase in connectivity between the right hippocampus and the ACC/mPFC in the benzodiazepine group than in the control group. Shown in *yellow*: overlap regions. (B) Partial correlation plot showing the negative association between PSQI score (y axis) improvement after four weeks of cupping treatment and the Fisher's Z values (x axis) of the significant cluster from A (red), including age, gender, and duration as covariates. (C) Partial correlation plot showing the negative association between PSQI score (y axis) improvement after four weeks of benzodiazepine treatment and the Fisher's Z values (x axis) of the significant cluster from A (green), including age, gender, and duration as covariates. Hipp, hippocampus; ACC, rostral anterior cingulate cortex; mPFC, medial prefrontal cortex.

showed a significant decrease in PSQI scores, but, there was no significant difference between the two treatment groups in PSQI score change. Our results are consistent with previous findings indicating that both cupping (Li and Zhang, 2013; LI et al., 2013; Zhang et al., 2010; Zhu et al., 2013) and pharmacologic (Brasure et al., 2016; Hall-Porter et al., 2010; Kuriyama and Tabata, 2017) interventions can significantly relieve the symptoms of insomnia. Therefore, our study suggests that non-drug treatment options, such as cupping, may be a promising alternative strategy for treating primary insomnia.

We found a significant GMV increase in the right hippocampus after 4 weeks of cupping intervention compared with controls. The observed brain morphometry changes in the hippocampus are in line with neurobiological models of insomnia that assume a dysfunction of brain areas associated with cognitive impairment. Literature suggests that daytime cognitive impairments and deficits in memory consolidation during sleep can be observed in individuals with PI, especially impairment in hippocampus-dependent memory consolidation (Mander et al., 2013; Suzanna and J Martin, 2007). For example, Riemann et al. (Riemann et al., 2007) reported a 15% reduction in bilateral hippocampal volumes in eight patients with primary insomnia in comparison to eight healthy sleepers. Joo et al. (Joo et al., 2014) also reported hippocampal volume loss in 27 patients with primary insomnia compared to 30 healthy sleepers across several hippocampal subfields. Our results suggest that successful non-pharmacological treatment may increase hippocampal volume to modulate the disturbed cognitive process.

The symptoms of insomnia are not limited to sleep and may best be summarized as a round-the-clock state of hyper-arousal, assuming an interplay between psychological and physiological factors in the etiology and perpetuation of primary insomnia (Perlis et al., 1997; Riemann et al., 2010). The hyper-aroused brain activity was changed in several core brain functional networks, including the default-mode network (DMN, hippocampus, ACC, and mPFC) and salience network (SN, ACC), which are associated with arousal. Specifically, it has been argued that the DMN may play a critical role in conscious awareness (Horovitz et al., 2009). Moreover, it has been shown that sleep deprivation, such as what might occur in insomnia, leads to aberrant stability and function of the DMN (De Havas et al., 2012; Gujar et al., 2014). The SN, the central network for detecting and filtering salient stimuli (Peters et al., 2016), has been demonstrated to play an important role in hyper-arousal and affective symptoms in patients with insomnia (Chen et al., 2014; Huang et al., 2012; Joo et al., 2013).

In the present study, we found that both non-pharmacological and pharmacological interventions significantly increased rsFC between the right hippocampus and left rACC/mPFC compared to the control group. We also found a negative association between insomnia symptom improvement and rsFC increase between the right hippocampus and left rACC/mPFC in both cupping and benzodiazepine groups. The ACC is a main node of the salience network (SN) and default mode network (DMN) and plays a critical role in the detection and screening of emotional stimuli (Uddin, 2015). Alterations in ACC volume (Winkelman et al., 2013) and abnormal ACC activity (Li et al., 2014; Wang et al., 2015a, 2015b) have been reported in PI in a previous study.

Literature suggests that the ACC also plays an important role in mediating dynamic interactions between large-scale brain networks involved in internal-oriented tasks (i.e. default mode network) and external-oriented tasks (i.e. central executive network, CEN) (Menon and Uddin, 2010). Li et al. demonstrated that insomnia patients exhibit increased connectivity between the superior parietal lobule and the right ACC, which are brain regions critical for spatial and verbal working memory (Li et al., 2014). Furthermore, affective and emotional symptoms appear to be closely connected with aberrations of the SN in patients with insomnia (Chen et al., 2014).

The mPFC is involved in memory, learning, and visuospatial tasks (Wen et al., 1999) and plays an active role in the generation of arousal and insomnia (Demontis et al., 1990). Previous studies have suggested that the insula and left mPFC are critical regions in maintaining sleep (Chuah et al., 2006; Koenigs et al., 2010). Moreover, another study reported that individuals with insomnia exhibited decreased activations in the DMN when performing working memory tasks (Drummond et al., 2013), and there appears to be a strong correlation between changes in the DMN circuitry and severity of insomnia (Regen et al., 2016). Similarly, changes in connectivity between the anterior DMN and SN may constitute an increase in top-down modulation of limbic hyperactivity, bottom-up interference of self-processing regions, or both, as has been previously argued to occur in depression (Mulders et al., 2015). As a result, some investigators have posited that insomnia is a network-based disorder (DMN & SN) (Chen et al., 2014). Taken together, our results suggest that both cupping and benzodiazepines may achieve treatment effects by modulating DMN and SN.

We did not find significant functional connectivity differences between the benzodiazepine and control groups using the left hippocampus as a seed at the threshold we set. As an exploratory analysis, we applied a relatively less conservative threshold of voxel-wise $p < .05$ with 100 continuous voxels and found greater connectivity between the left hippocampus and bilateral ACC/mPFC. More studies with larger sample sizes are needed to validate our finding.

There are several limitations to this study. First, the treatment only lasted four weeks. Thus, the results obtained only represent short-term to mid-term effects. Further study is needed to evaluate the long-term effects of non-pharmacological and pharmacological interventions. Second, the dropout rate in each group was relatively high; however, we would like to emphasize that the reasons for dropout did not seem to be associated with treatment response. Third, we only recruited patients with PI for the benzodiazepine and cupping treatments, so there were no healthy controls (HCs) in this study. This prevents us from exploring modulation effects of different interventions on the disrupted changes in brain structure and function of PI patients as compared to HCs. Fourth, we did not systemically collect the side effects that occurred during cupping or benzodiazepine treatment. Further research is needed to record any side effects corresponding with or temporally related to treatment in a diary booklet. Finally, the sample size is relatively small, and studies with larger sample sizes are needed to further validate our findings.

5. Conclusion

We found that both cupping and benzodiazepines can significantly modulate hippocampal and rACC/mPFC rsFC. The strengthened functional connection was significantly associated with the therapeutic effects of treatment. Our findings demonstrate the potential of cupping in the treatment of insomnia.

Declaration of interest

JK has a disclosure to report (holding equity in a startup company, MNT, and a pending patent to develop a new brain stimulation device) but declares no conflict of interest. All other authors declare no competing interests.

Contributors

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Ethical statement

The experiment was approved by the Institutional Review Board of the Affiliated Hospital of Chengdu University of Traditional Chinese Medicine (CDUTCM) and was registered at www.chictr.org.cn (No. ChiCTR-ICR-15006021). All subjects signed a written consent form and agreed to allow their data to be analyzed.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nicl.2019.101745.

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