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Frequency-dependent changes in local intrinsic oscillations in chronic primary insomnia: A study of the amplitude of low-frequency fluctuations in the resting state

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ABSTRACT

New neuroimaging techniques have led to significant advancements in our understanding of cerebral mechanisms of primary insomnia. However, the neuronal low-frequency oscillation remains largely uncharacterized in chronic primary insomnia (CPI). In this study, the amplitude of low-frequency fluctuation (ALFF), a data-driven method based on resting-state functional MRI, was used to examine local intrinsic activity in 27 patients with CPI and 27 age-, sex-, and education-matched healthy controls. We examined neural activity in two frequency bands, slow-4 (between 0.027 and 0.073 Hz) and slow-5 (0.010–0.027 Hz), because blood-oxygen level dependent (BOLD) fluctuations in different low-frequency bands may present different neurophysiological manifestations that pertain to a spatiotemporal organization. The ALFF associated with the primary disease effect was widely distributed in the cerebellum posterior lobe (CPL), dorsal and ventral prefrontal cortex, anterior cingulate cortex, precuneus, somatosensory cortex, and several default-mode sub-regions. Several brain regions (i.e., the right cerebellum, anterior lobe, and left putamen) exhibited an interaction between the frequency band and patient group. In the slow-5 band, increased ALFF of the right postcentral gyrus/inferior parietal lobule (PoCG/IPL) was enhanced in association with the sleep quality ($\rho = 0.414$, $P = 0.044$) and anxiety index ($\rho = 0.406$, $P = 0.049$) of the CPI patients. These findings suggest that during chronic insomnia, the intrinsic functional plasticity primarily responds to the hyperarousal state, which is the loss of inhibition in sensory-informational processing. Our findings regarding an abnormal sensory input and intrinsic processing mechanism might provide novel insight into the pathophysiology of CPI. Furthermore, the frequency factor should be taken into consideration when exploring ALFF-related clinical manifestations.

1. Introduction

Insomnia is a common health complaint that includes difficulty falling asleep and staying asleep, as well as feelings of non-restorative sleep. Insomnia is associated with daytime fatigue, mood disruption, and cognitive impairment. Approximately 70% of individuals with insomnia display persistent symptoms for more than three months, which is defined as chronic primary insomnia (CPI) (Ohayon and Roth, 2003). However, relatively few studies have used neuroimaging techniques to examine the physiology of CPI such as the cerebral mechanisms underlying the pathogenesis of insomnia and the neural correlates of insomnia symptoms. A clearer understanding of the pathophysiological mechanisms would help achieve further advances in the prevention and treatment of the condition.

Although results from structural imaging studies (morphometric magnetic resonance imaging [MRI]) vary widely, it appears that

Abbreviations: ACC, anterior cingulate cortex; ALFF, amplitude of low-frequency fluctuation; ANOVA, analysis of variance; CPI, chronic primary insomnia; CPL, cerebellum posterior lobe; FC, functional connectivity; fMRI, functional MRI; fO/AI, frontal operculum/anterior insula; Fus/CAL, fusiform gyrus/cerebellum anterior lobe; HC, healthy control; MFG/SFG, middle/superior frontal gyrus; MOG, middle occipital gyrus; mPFC, medial prefrontal gyrus; MRI, magnetic resonance imaging; mTL, medial temporal lobe; PCC, posterior cingulate cortex; PCUN, precuneus; PoCG/IPL, postcentral gyrus/inferior parietal lobule; PSQI, Pittsburgh Sleep Quality Index; rs-fMRI, resting-state fMRI; SPM, statistical parametric mapping; SPECT, single-photon emission computed tomography; STG, superior temporal gyrus; STAI-s, State Trait Anxiety Inventory-state; STAI-t, State Trait Anxiety Inventory-trait.

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neuroanatomical alterations mostly occur in the hippocampus, anterior cingulate cortex (ACC), and orbitofrontal cortex (Altena et al., 2010; Joo et al., 2013; O'Byrne et al., 2014). Functional imaging studies, including single-photon emission computed tomography (SPECT) (Smith et al., 2002), positron emission tomography (PET) (Bonnet and Arand, 1995), and functional MRI (fMRI) (Drummond et al., 2013), have provided evidence of a hyperarousal state in sensory information processing, as well as cognitive and emotional abnormalities in primary insomnia. However, the cerebral functional plasticity in CPI patients is not well understood, and it is not clear how chronic insomnia impacts the intrinsic functional connectivity (FC) architecture and the neural correlates of insomnia symptoms. Resting-state fMRI (rs-fMRI) is a novel technique that provides new opportunities to identify broadly connected functional networks or modalities in patients with CPI. Alterations in connectivity, including decreased connectivity in emotional circuits (Huang et al., 2012), increased connectivity in sensory and motor regions (Killgore et al., 2013; Li et al., 2014b), increased connectivity between the insula and salience networks (Chen et al., 2014) and altered parietal-frontal connectivity related to working memory (Li et al., 2014b) or altered local activity in regional homogeneity (ReHo) (Dai et al., 2014; Wang et al., 2015), have previously been described in patients with insomnia. Nevertheless, previous rs-fMRI studies in patients with insomnia have typically utilized a low-frequency band of 0.01–0.08 Hz to measure co-activity within predefined regions (FC analyses) or local functional homogeneity within a small region (ReHo analyses (Dai et al., 2014)) and have ignored the frequency property of rs-fMRI signals.

In the measurement of the frequency property, the amplitude of low-frequency fluctuation (ALFF) measures the absolute strength or intensity of low-frequency oscillations of BOLD fluctuations, with high test-retest reliability (Zuo and Xing, 2014). Intriguingly, it has been demonstrated that different spatial distributions of ALFF in distinct frequency bands, such slow-4 (0.027 – 0.073 Hz) ALFF, were most robust in the subcortical and prefrontal regions (Zuo et al., 2010). In an fMRI study, the ALFF was divided into five frequency bands, including slow-6 (0–0.01 Hz), slow-5 (0.01 – 0.027 Hz), slow-4 (0.027 – 0.073 Hz), slow-3 (0.073–0.198 Hz) and slow-2 (0.198 – 0.25 Hz) (Zuo et al., 2010). It has been indicated that the differential neurophysiological manifestations that underlie distinct frequencies may arise from the neuronal input selection and plasticity. For example, the dynamic oscillations of high-frequency from the discharge of pyramidal cells in the receptive field “enslaves" the basket cells through resonance tuning; however, the exact mechanism remains poorly understood (Buzsáki and Draguhn, 2004). Previous studies of electroencephalography (EEG) have demonstrated reduced alpha power (Freedman, 1986) and gamma ratio power (Chen et al., 2014) during wakefulness in insomnia, and the gamma ratio power has been associated with the rs-fMRI signal of the anterior insula in insomniacs (Chen et al., 2014), which indicates a specific prefrontal activity pattern in different spectral bands of EEG (Chen et al., 2014; Perrier et al., 2015).

Regarding alterations relevant to insomnia, a recent study investigated the regional brain activity (ALFF) changes in both male and female CPI patients (Dai et al., 2016); however, it remains largely unknown whether the different alterations are frequency-band specific or frequency dependent on the regional properties of spontaneous activity in CPI patients. In the current study, we will clarify the alterations in the ALFF across different frequencies in CPI and healthy control (HC) groups. We hypothesized that the distinct activity pattern in different low frequency bands were relevant to insomnia and that these insomnia-related alterations exhibited significant frequency-dependence. Motivated by previous work (Gao et al., 2015; Han et al., 2011; Zhang et al., 2015), this study utilized ALFF to examine regional intrinsic neural activity at different frequency bands in CPI patients. The exact frequency bins that were used in this study mainly focus on slow-5 and slow-4 because these frequency bins belong to the typical low-frequency bands that are considered to primarily contribute physiological manifestations in blood-oxygen level dependent (BOLD) fluctuation analyses. In addition, the relationships between the regions that yielded significant differences between the groups (CPI and HC groups) with regard to sleep quality were also investigated.

2. Material and methods

2.1. Subjects

All of the subjects provided their written informed consent to participate in the study. The CPI patients and age- and sex-matched HC subjects were recruited at our hospital and from the local community from May 2012 to July 2013. The inclusion criteria for the patient group included (1) meeting the diagnostic criteria for primary insomnia according to the Diagnostic and Statistical Manual of Mental Disorders, version 4 (DSM-IV); (2) duration of insomnia ≥1 year; (3) without medical treatment; (4) age 25–65 years; and (5) right-handed. All of the patients reported difficulty initiating or maintaining sleep and/or reported nonrestorative sleep with resulting daytime distress or dysfunction that was not attributable to another medical or psychiatric disorder. The exclusion criteria for both the patients and controls were as follows: (1) clinical evidence of any moderate-to-severe sleep disorder other than insomnia (e.g., obstructive sleep apnea (OSA), restless legs syndrome); (2) abnormal sleep-wake rhythms; (3) hypertension, diabetes, or heart or respiratory diseases; (4) history of cerebrovascular disease or any other neurological (neurodegenerative diseases, epilepsy, head injury) or psychiatric diseases (psychosis, current depression); (5) alcohol or illicit drug abuse or current intake of psychoactive medications; and (6) contraindications for MRI, such as claustrophobia, metallic implants or devices in the body or a structural lesion detected on the brain MRI. Each patient presented maximum displacement of the rs-fMRI data in any of the cardinal directions (x, y, z) < 2 mm and a maximum spin (x, y, z) < 2° (see the rs-fMRI data preprocessing section below).

Finally, 27 right-handed, treatment-naive CPI patients and 27 age-, sex-, and education-matched HC subjects were selected from all of the participants. Of note, these CPI patients have been reported in our previous study using ReHo analysis (as a master's thesis in Chinese, http://cdmd.cnki.com.cn/Article/CDMD-10411-1014116735.htm), which was the first ALFF analysis of these patients. The current study is a completely independent study and does not contain the exact data or the same patients previously used in the Dai et al. study (Dai et al., 2016). All participants were instructed not to consume caffeine, alcohol, central nervous system (CNS)-activating agents, or any other psychoactive substances for 48 h before the rs-fMRI study. The present study was performed according to approved guidelines and conducted in compliance with the principles of the Declaration of Helsinki. This study was approved by the Medical Research Ethics Committee and the Institutional Review Board of The First Affiliated Hospital of Nanchang University.

2.2. Evaluation of subjective sleep quality and mood status

All of the subjects underwent a clinical evaluation, including the Pittsburgh Sleep Quality Index (PSQI) for sleep quality, the Beck Depression Inventory-II (BDI-II) for depression, and the State Trait Anxiety Inventory-state (STAI-s) and State Trait Anxiety Inventory-trait (STAI-t) for anxiety, which are all common assessments used to identify emotional disorders.

2.3. MRI data acquisition

The subjects were scanned using a Trio 3.0-T scanner system and an 8-channel head coil (Siemens, Erlangen, Germany). The subjects were instructed to maintain their eyes closed and remain resting on the foam pads to minimize head motion, and they were told not to think

of anything in particular and to stay awake. (1) A standard T_2*-weighted gradient echo sequence was established to acquire rs-fMRI images. The scan parameters were as follows: repetition time/echo time = 2000/30 ms; field of view = 220 × 220 mm; matrix = 64 × 64; and interleaved axial slices = 30 at a thickness of 4 mm with an interslice gap of 1.2 mm. This acquisition sequence generated 240 volumes in 8 min. (2) A 3-dimensional, high-resolution T_1-weighted sequence was established for anatomical images: repetition time/echo time = 1900 ms/2.26 ms; matrix = 240 × 256; field of view = 215 × 230 mm; sagittal slices = 176 at a thickness of 1.0 mm with no gap. (3) A conventional T_2-weighted and fluid-attenuated inversion recovery (FLAIR) imaging protocol was used to exclusively diagnose the patients. At the end of the scanning sessions, the subjects confirmed that they had not fallen asleep with an Epworth sleepiness scale (ESS) questionnaire. All scans were performed between 18:00 and 20:00, according to the time scheme of our MRI lab.

2.4. rs-fMRI data preprocessing

The preprocessing of rs-fMRI images was performed using a toolbox for Data Processing & Analysis of Brain Imaging (Yan and Zang, 2010) (http://rfmri.org/dpabi) based on statistical parametric mapping (SPM8, http://www.fil.ion.ucl.ac.uk/spm/software/spm8/), which was run on MATLAB 8.4.0 (Mathworks, Natick, MA, USA). This preprocessing was performed with the following steps: (1) the first 10 images from each subject were discarded during data acquisition to eliminate magnetic saturation effects and enable the subjects to adapt the scanning noise of each session; (2) the remaining 230 images were corrected for slice timing and voxel-specific head motion calculations and corrections to adjust the time series of the images (head motion was <2 mm of translation along any axis and <2.0° of angular rotation along any axis); (3) the group differences in head motion were evaluated among the CPI patients (n = 27) and HC subjects (n = 27) according to the frame-wise displacement (FD) criteria described by Van Dijk et al. (Van Dijk et al., 2012). The results indicated that the two groups did not display significant differences in head motion (one-way analysis of variance with Bonferroni-corrected post-hoc two-sample *t*-tests, $P = 0.759$; Table 1); (4) the high-resolution individual T_1WI images were co-registered to the mean functional image after motion correction using a linear transformation; (5) the co-registered functional images were normalized to the Montreal Neurological Institute (MNI) space with 3 × 3 × 3 mm^3 re-sampling; (6) spatial smoothing was performed with an isotropic Gaussian kernel with a 6 mm full-width-half-maximum Gaussian kernel; (7) linear trends within the time series were removed; (8) nuisance variables were extracted from the rs-fMRI data, which included a ventricular signal averaged from the ventricular regions of interest in a preset standard template, a white matter signal averaged from white matter regions of interest, a whole-brain signal averaged across the whole brain, and 24 head realignment parameters obtained by rigid-body head motion correction. Nuisance linear regression was performed with the white matter, cerebrospinal fluid, global signal, six head motion parameters, 6 head motion parameters at one time point earlier, and the 12 corresponding squared items (Friston 24-parameter model) as covariates.

Table 1
Comparison of the demographic and neuropsychological data for the CPI and HC groups.

Characteristic	Patients with CPI	Healthy controls	*P*-values
	Mean (SD)	Mean (SD)	
Gender (M/F)	17/10	17/10	1.00
Age (y)	42.59 (11.59)	40.92 (11.46)	0.598
Education (y)	9.52 (3.04)	10.52 (4.17)	0.319
Duration of insomnia (y)	10.98 (8.85)	n/a (n/a)	n/a
STAI-s	28.07 (4.17)	26.22 (8.14)	0.298
STAI-t	32.26(4.81)	29.07 (10.10)	0.145
BDI-II	6.15 (5.30)	4.92(1.59)	0.260
PSQI	13.29 (2.54)	0.85(1.06)	<0.0001
Mean head motion*	0.043(0.025)	0.046(0.041)	0.759

Note: BDI, Beck Depression Inventory; CPI, chronic primary insomnia; F, female; M, male; n/a, not available; PSQI, Pittsburgh Sleep Quality Index; SD, standard deviation; STAI-s, the State Trait Anxiety Inventory-state; STAI-t, the State Trait Anxiety Inventory-trait; y, years.
*Head motions were evaluated according to the frame-wise displacement (FD) criteria described by Van Dijk et al. (2012).

2.5. ALFF analysis

The ALFF was calculated using the Data Processing & Analysis of Brain Imaging toolbox with the following steps: (1) The full frequency range (0–0.25 Hz) of the rs-fMRI signal was divided into five different bands according to the Buzsáki framework (Buzsáki and Draguhn, 2004): (slow-6: 0–0.01 Hz; slow-5: 0.01–0.027 Hz; slow-4: 0.027–0.073 Hz; slow-3: 0.073–0.167 Hz; and slow-2: 0.167–0.25 Hz). (2) For a given voxel, the time series was first converted to the each frequency domain using a fast Fourier transform procedure. (3) The square root of the power spectrum was calculated and then averaged across a predefined frequency interval, and this averaged square root was termed the ALFF at the given voxel. (4) The ALFF was standardized by dividing the participant's whole-brain voxel average ALFF (slow-5: 0.560 ± 0.1260 vs 0.576 ± 0.1493, respectively, $P = 0.669$; slow-4: 0.432 ± 0.0822 vs 0.445 ± 0.1065, respectively, $P = 0.626$) to reduce the global effects of variability across the participants, which measures the absolute strength or intensity of the spontaneous low-frequency oscillations.

2.6. Statistical analysis

In this study, we calculated the ALFF in the slow-5 and slow-4 bands as the main results. We also report the ALFF results of the other 5 frequency bands as referential results, because slow-6, slow-3, and slow-2 have typically been were discarded as physiological noise, despite several studies that have analyzed the 5 frequency bands in brain disorders, including chronic somatic pain (Malinen et al., 2010), visceral pain (Hong et al., 2013), and late-onset depression (Yue et al., 2015).

To investigate the main effects of the frequency band, group, and their interactions, we performed a two-way ANOVA (double-factor, 2 × 2) using the SPM8 software program (Wellcome Institute of Cognitive Neurology, London, UK) within a standard (Montreal Neurological Institute space) gray matter mask, voxel by voxel. The group (the CPI patients vs. the HC subjects) served as a between-subject factor, the frequency band (slow-5 and slow-4) was an independent-measures factor, and gender, age, and the mean FD was covariates (Gao et al., 2015; Han et al., 2011; Zhang et al., 2015). Further post-hoc two-sample *t*-tests were performed for group comparison of the slow-5 band and slow-4 band results. In the analysis of the referential results, we performed an ANOVA (flexible factorial design, 2 × 5) with the group (CPI and HC) as a between-subject factor and the frequency band (slow-2 to slow-6) as an independent-measure using SPM8 within a standard gray matter mask. All significance tests were conducted at a family-wise error (FWE) rate threshold of $P = 0.05$ derived using Monte Carlo simulation (AlphaSim; single voxel $p = 0.05$, FWHM = 6 mm, 10,000 simulations, using the standard gray matter mask [47,508 voxels]) (http://www.restfmri.net/) (Ledberg et al., 1998).

A further correlative analysis between the ALFF value for significantly different brain areas and neuropsychological performance was then performed on the CPI groups by extracting significantly different frequencies between the groups. A partial correlation analysis was performed to examine the relationships between an abnormal ALFF and standardized neuropsychological performance scores using the SPSS 13.0 software program (SPSS, Inc., Chicago, IL, USA).

3. Results

3.1. Evaluation and demographic results

Significant differences in age, gender, or education level were not observed between the CPI and HC groups. Compared with the HC subjects, the CPI patients had significantly higher scores on the PSQI ($t = 23.49$, $P < 0.0001$), whereas significant differences were not observed for the scores of Beck Depression Inventory-II ($t = 1.148$, $P = 0.260$), STAI-s ($t = 1.052$, $P = 0.298$), or STAI-t ($t = 1.479$, $P = 0.145$) (see Table 1).

3.2. ALFF results

3.2.1. Main effect for group factor

Specific brain regions exhibited significant main effects for group in which the CPI patients had lower ALFF values than the HC subjects for the right cerebellar posterior lobe (CPL), left CPL, right frontal operculum/anterior insula (fO/AI), right middle/superior frontal gyrus (MFG/SFG), left MFG, bilateral medial prefrontal gyrus (mPFC)/ACC, and bilateral precuneus (PCUN). The brain regions showing a significant main effect for group in which the CPI patients had higher ALFF values than the HC subjects included the left fusiform gyrus/cerebellar anterior lobe (Fus/CAL), left medial temporal lobes (mTL), bilateral retrosplenial cortices (RSC), left middle occipital gyrus (MOG), and right postcentral gyrus/inferior parietal lobule (PoCG/IPL) (Fig. 1, Table 2). A single-voxel threshold for the map resulting from the *t*-test was set at 0.05, and a minimum cluster size of 3024 mm^3 (112 voxels) was used to correct for multiple comparisons, as determined by a Monte Carlo simulation.

3.2.2. Main effect for frequency band factor

The two-way ANOVA results of the effect of the frequency band are summarized in Fig.2 and Table A.1. Compared with the slow-5 band, the slow-4 band exhibited significantly increased ALFF in the left superior temporal gyrus (STG), right STG, and bilateral thalami/caudate/CAL, whereas it was decreased in the right CPL, right fusiform gyrus/ITG/medial temporal lobe (mTL), left ITG/mTL, and bilateral medial prefrontal cortex (mPFC)/inferior frontal gyrus/rectus/anterior cingulate cortex ($P < 0.05$, AlphaSim corrected).

Table 2
Significant differences in the ALFF based on the main effects of disease between the CPI and HC groups.

Brain regions	BA	Peak T-scores	MNI coordinates			Cluster size (voxels)
			x	y	z	
CPI < HC						
right CPL		−4.35	12	−81	−21	277
left CPL		−3.58	−36	−75	−30	190
right fO/AI	38,44	−3.92	51	18	−12	114
right MFG/SFG	10	−4.28	27	51	24	134
left MFG	10	−3.99	−30	51	6	168
bilateral mPFC/ACC	8,9,10,24,33	−4.77	9	33	48	411
bilateral PCUN	5,7	−4.03	−3	−51	54	117
CPI > HC						
left Fus/CAL		4.04	−45	−33	−18	173
left mTL	20,21	4.75	−57	−3	−27	132
bilateral RSC	29,30	4.94	−6	−48	6	133
left MOG	18	3.56	−33	−87	33	115
right PoCG/IPL	2,40	5.56	54	−27	39	168

Note: ALFF, amplitude of low-frequency fluctuation; ACC, anterior cingulate cortex; AI, anterior insula; BA, Brodmann's area; CAL, cerebellar anterior lobe; CPI, chronic primary insomnia; CPL, cerebellar posterior lobe; fO, frontal operculum; HC, healthy controls; IPL, inferior parietal lobule; MFG, Fus, fusiform gyrus; MFG, middle frontal gyrus; mPFC, medial prefrontal gyrus; mTL, medial temporal lobes; MNI, Montreal Neurological Institute; MOG, middle occipital gyrus; PoCG, postcentral gyrus; PCUN, precuneus; RSC, retrosplenial cortex and SFG, superior frontal gyrus; the same abbreviations are used for all figures and tables.

3.2.3. Interactions between the group and frequency band

A significant interaction was identified in the right CAL and left putamen between the frequency band and group (Fig. A.1). Further post-hoc two-sample t tests indicated that the ALFF significantly increased in the right PoCG/IPL in the slow-5 band in the CPI patients and decreased in the bilateral mSFG in the slow-4 band ($P < 0.05$, with AlphaSim correction) (Fig. 3, Table 3).

As a referential result, we report the frequency-dependent ALFF results of 5 frequency bands (slow-2 to slow-6) in the Supplementary

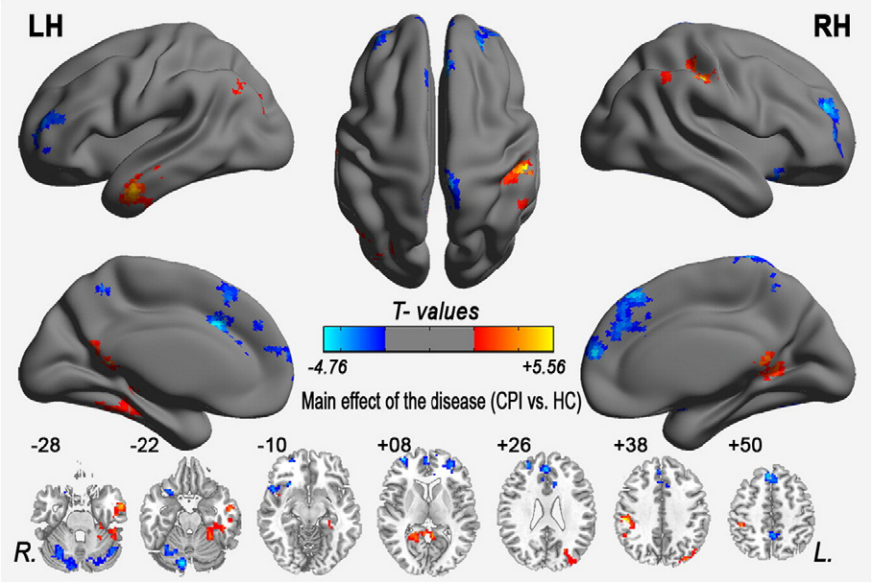

Fig. 1. Maps of the main effect of the disease between the CPI and HC groups. A single-voxel threshold for the map resulting from the *t*-test was set at 0.05, and a minimum cluster size of 3024 mm^3 (112 voxels) was used to correct for multiple comparisons by the Monte Carlo simulation. Hot colors indicate that the CPI group had increased ALFF compared with the HC group; cold colors indicate the opposite.

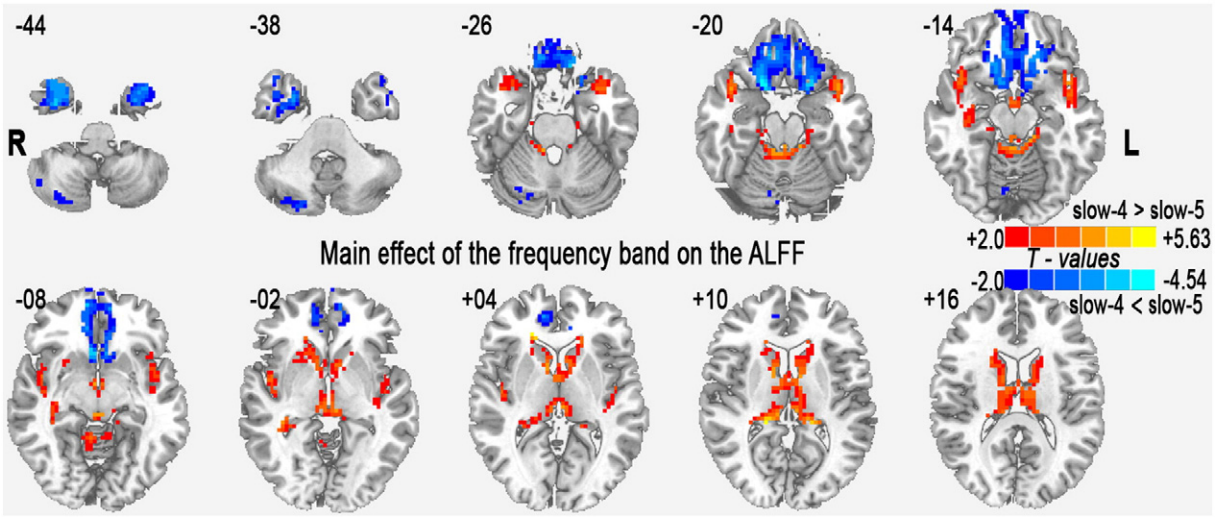

Fig. 2. Main effect of the frequency band on the ALFF. Differences in the ALFF values between the slow-4 and slow-5 frequency bands, based on two-way ANOVA (double-factor), $P < 0.05$, with an AlphaSim correction and a minimum cluster size of 3024 mm^3 (112 voxels). Hot and cold colors indicate higher and lower ALFF values in the slow-4 band relative to the slow-5 band, respectively. L = left; R = right.

Materials (Figs. A.2 and A.3). In the slow-2 to slow-6 analyses, their alteration patterns were similar in the main effect of the group factor.

3.3. Relationships between abnormal ALFF values and neuropsychological assessments

For correlation analysis, the ALFF value of CPI patients was extracted from significantly different brain areas between groups in different frequency bands (slow-5, slow-4) and from the main effect for group factor. Correlations between the abnormal ALFF values and neuropsychological assessments were calculated for the CPI group. In the CPI patients, increased ALFF in the right PoCG/IPL was positively correlated with the PSQI score ($P = 0.044$) and the STAI-s score ($P = 0.049$) in the slow-5 band (Fig. 4). However, significant relationships were not identified between the abnormal ALFF values in the slow-4 band, the ALFF values in the main effect for group factor and the neuropsychological assessments (P: 0.052 to 0.994).

4. Discussion

We investigated ALFF alteration in CPI patients in different frequency bands (slow-4 and slow-5) via rs-fMRI analysis. First, the CPI patients showed several significant differences in ALFF between two groups. A significant interaction between the frequency band and group was also identified in the right CAL and left putamen. Moreover, with regard to behavior, a CPI-related increase in the ALFF in the right PoCG/IPL, which was related to poor sleep quality (PSQI) and objective anxiety (STAI-s), was identified only in the slow-5 band. Our findings suggest that our understanding of spontaneous neural activity in rs-fMRI analysis of CPI patients is improved when the frequency is considered.

4.1. Alterations of the ALFF in CPI patients

4.1.1. Decreased ALFF in CPI patients

The ALFF has been proposed to assess the amplitude of local spontaneous activity in healthy individuals (Buzsividualsneous CPI2004; Zuo

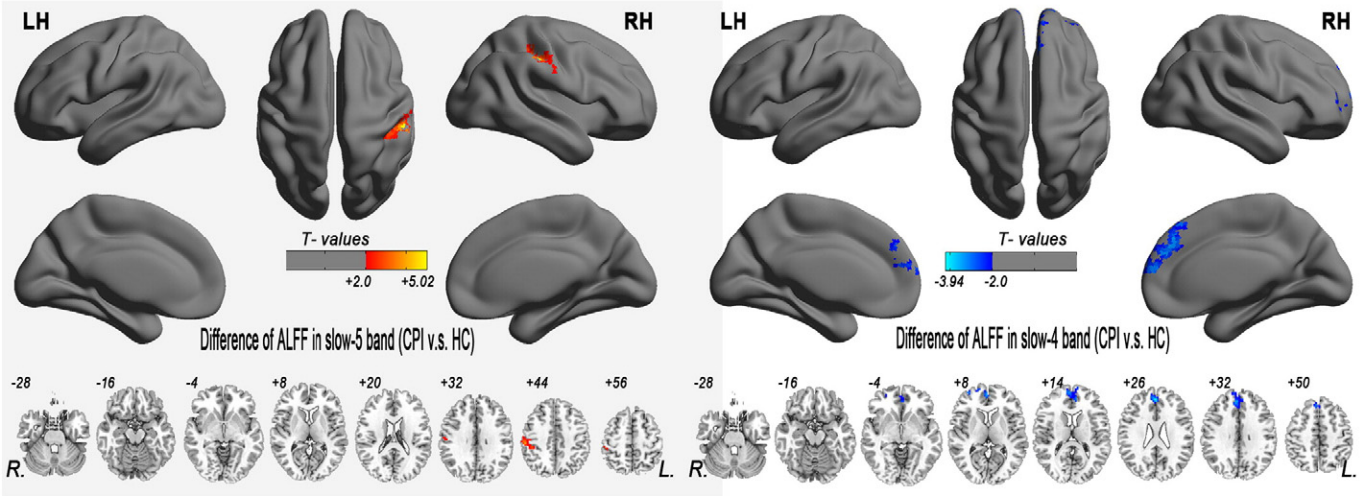

Fig. 3. Differences in the amplitude of low-frequency fluctuations (ALFF) between the patient groups in two low-frequency bands. Right: Differences in the ALFF between the CPI patients and healthy controls in the slow-5 band. B: Differences in the ALFF between the CPI patients and healthy controls in the slow-4 band (two-sample t-test; $P < 0.05$, with AlphaSim correction).

Table 3
Significant interaction effects between the frequency band (slow-4 and slow-5) and group by a two-way ANOVA (double-factor) and a post hoc test.

Brain regions	BA	Peak *t*-scores	MNI coordinates			Cluster size (voxels)
			x	y	z	
In the slow-5 band						
CPI < HC						
None						
CPI > HC						
Right PoCG/IPL	2,40	5.02	54	−27	42	131
In the slow-4 band						
CPI < HC						
Bilateral mSFG	9,10	−3.94	3	45	27	352
CPI > HC						
None						

Note: T statistical value of peak voxel showing ALFF differences. All the clusters had $P < 0.05$, with AlphaSim correction and a minimum cluster size of 3024 mm^3 (112 voxels).

et al., 2010) and pathological brains (Yu et al., 2014; Zang et al., 2007; Zhou et al., 2014). The neurophysiological mechanisms that underlie distinct frequency-band properties or specific classes of oscillations may be caused by different neuronal origins, differences in cytoarchitecture, or links to specific neural processes (Buzs links to specifi2004; Penttonen, 2003), including input selection, plasticity, binding, and consolidation (Zuo et al., 2010); however, although the exact mechanism remains poorly understood. In this study, the CPI patients had several decreased-ALFF regions, including several DMN subregions (bilateral mPFC/ACC and PCUN), the prefrontal cortex (left MFG and right MFG/SFG), integrated regions (right fO/AI), right and left CPL. These findings indicate that chronic insomnia may disrupt the amplitude patterns of cortical oscillatory activity.

The DMN is composed of brain regions that are active when the brain is not otherwise engaged in goal-oriented behavior. In CPI patients, alterations in mPFC/ACC and PCUN have been reported in several studies, including hypoperfusion and hypometabolism in this region (Nofzinger et al., 2006) by positron emission tomography (Smith et al., 2002) and SPECT (Smith et al., 2005). The inability to deactivate task-irrelevant brain regions during performance (Altena et al., 2008; Drummond et al., 2013), even in female CPI patients, is also correlated with lower ALFF in mPFC/ACC (Dai et al., 2016). Our results agree with a very recent study (Dai et al., 2016) and suggest that the dysfunction of self-relevant, affective decisions in CPI patients is affected by decreased activity in the mPFC/ACC and PCUN (dorsal midline core of DMN).

Structural studies suggest that the fO/AI has anatomical atrophy in primary insomnia (Altena et al., 2010; Joo et al., 2013; O'Byrne et al., 2014), which supports our findings. Previous studies have demonstrated different FC patterns between the insula and other regions, for example, hypo-connectivity between the AI and the parietal cortex (Li et al., 2014b) and amygdala (Huang et al., 2012) and hyper-connectivity between the insula and salience networks (Chen et al., 2014). Considered together, one interpretation of the FC findings is that an arousal-promoting effect is involved in the hyperarousal state in these patients. This finding provides novel insight into insular function.

Numerous studies have reported that the prefrontal cortex is involved in emotional processing. Indeed, decreased gray matter volumes in CPI patients have been reported in the middle or superior frontal gyrus in patients with CPI (Joo et al., 2013), resulting in poorer working memory performance (Drummond et al., 2013; Joo et al., 2013). Overall, decreased ALFF in the SFG and MFG may result in emotional syndromes. The CPL plays an important role in fine motor coordination, specifically the inhibition of involuntary movement. Reduced ALFF in the CPL suggest as loss of inhibition in internal spontaneous motor-related activity.

Considered together, these results suggest that chronic insomnia is closely linked to a disrupted amplitude pattern of cortical oscillatory activity and that the intrinsic activity is associated with chronic sleep loss, both cognitively and emotionally.

4.1.2. Increased ALFF in CPI patients

In this study, we also observed increased ALFF values for neuronal activity in CPI patients predominantly in several posterior regions of the DMN (the RSC, IPL and mTL), secondary visual cortex (MOG) and somatosensory cortex (PoCG). CPI patients exhibit sex differences in increased ALFF, indicating that they share analogous excessive hyperarousal mechanisms during daytime (Dai et al., 2016). Similarly, compared with HC subjects, CPI patients have increased connectivity (Killgore et al., 2013) and low-frequency BOLD fluctuations in sensory processing regions during resting wakefulness, which is consistent with a hyperarousal state and is associated with difficulty falling asleep (Killgore et al., 2013; Riemann et al., 2010). Hyperarousal processes play a key role in the pathophysiology of primary insomnia. Moreover, in a recent study of sleep-deprived subjects, the participants showed increased ("compensatory") task-related activation and reduced deactivation in part of the DMN (Drummond et al., 2013). This reduced deactivation was theorized to reflect the greater degree of externally oriented processing engaged in by insomniacs at even the lowest level of task difficulty. The increased ALFF observed in the present study in the posterior DMN, including the mTL for memory and PCC with adjacent ventral PCUN for integration, participates in hyperarousal

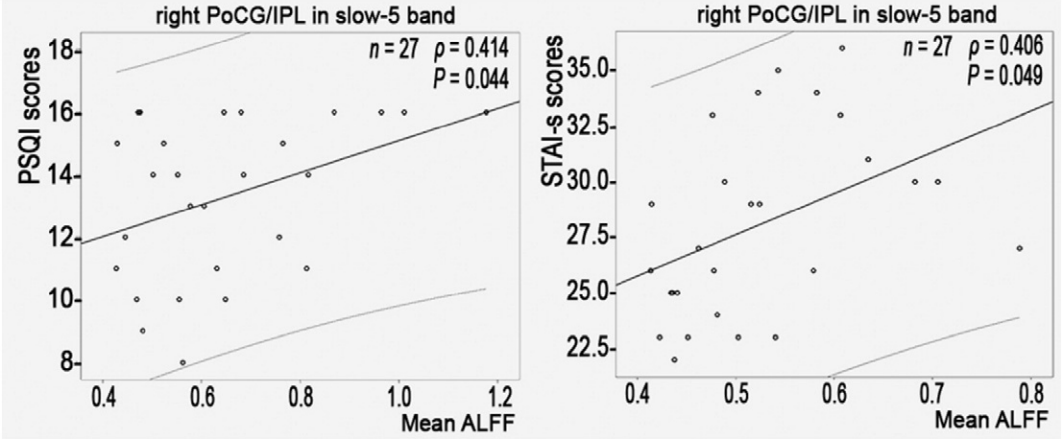

Fig. 4. Abnormal clusters of the ALFF stratified by the largest contribution were significantly correlated with the neuropsychological assessments in CPI patients ($P < 0.05$, with bootstrapping statistics). ALFF = amplitude of low-frequency fluctuation; IPL = inferior parietal lobule; PoCG = postcentral gyrus; PSQI = Pittsburgh Sleep Quality Index; STAI-s = State Trait Anxiety Inventory-state.

processes associated with resting wakefulness. However, decreased connectivity within the DMN has been demonstrated to occur as a result of sleep loss/deprivation (Basner et al., 2013; McKenna and Eyler, 2012), despite a significant increase in the fluctuation level of the BOLD signal during light sleep (Basner et al., 2013). Further work is required to clarify the distribution of ALFF changes throughout the DMN and the DMN's connectivity in CPI patients.

4.2. Differences in the ALFF between frequency bands

We demonstrated that abnormalities in the ALFF in the CPI patients were associated with specific frequency bands, which indicates that frequency bands should be taken into account when measuring the intrinsic local brain activity of CPI patients. Compared with the slow-5 band, the frequency effect analysis indicated a higher ALFF in the slow-4 band in the left and right STG, as well as individual clusters in the subcortical regions (bilateral thalami/caudate/CAL) (Fig. 2, Table 2). Increasing evidence regarding ALFF has indicated that the slow-4 band exhibits an increased ALFF compared with the slow-5 band in subcortical regions(Gao et al., 2015; Zhang et al., 2015; Zuo et al., 2010), as previously suggested by spontaneous electrophysiological recordings (Ruskin et al., 2001). In this study, we also identified a higher ALFF in the right CPL, right Fus/ITG/mTL, left ITG/mTL, and bilateral mPFC/IFG/rectus/anterior cingulate cortex in the slow-5 band. These increased ALFF regions include several default-mode network (DMN) sub-regions (mPFC and mTL), and the findings are consistent with previous reports that the slow-5 band has a higher ALFF in the frontal cortex and DMN sub-regions (Gao et al., 2015; Zuo et al., 2010).

The possibility that physiological (cardio, respiratory) or other structure-related noise may affect brain regions should be considered (Yuan et al., 2014; Zuo et al., 2013). However, in general, we assume that the noise may be a random factor in the CPI and HC groups; thus, there should not be significant differences between the CPI and HC groups, even when the noise effect was compared between the slow-4 and slow-5 bands. In this study, we identified two interactional regions between the frequency band (slow-4 band vs. slow-5 band) and the disease (CPI vs. HC) on the ALFF (Fig. A.1), which indicates a significant difference in the detected frequency-specific changes. The effect of this interaction was more likely to be caused by slow-4 and slow-5 associated neural activity; however, noise-related differences between the two groups cannot be eliminated.

4.3. Frequency-specific changes in the ALFF in CPI patients

In this study, the ALFF in the CPI patients increased in the right PoCG/IPL in the slow-5 band but decreased in the bilateral mSFG in the slow-4 band, reflecting that the pattern of local intrinsic brain activity is sensitive to specific frequency bands and that we should take the frequency bands into account when measuring the ALFF of CPI patients. Previous studies demonstrated frequency-specific alterations in amplitude patterns linked to sleep deprivation (Gao et al., 2015) and other diseases, such as amnestic mild cognitive impairment patients (Han et al., 2011), Parkinson's disease (Hou et al., 2014) and subcortical ischemic vascular disease (Li et al., 2014a). Zuo et al. (Zuo et al., 2010) demonstrated that ALFF has different properties and physiological functions in different low-frequency bands (slow-5 and slow-4 bands). The neurophysiological mechanisms underlying the distinct frequency-band properties or specific classes of oscillations may be caused by different neuronal origins, differences in cytoarchitecture, or links to specific neural processes (Buzsáki and Draguhn, 2004; Penttonen, 2003). Thus, our results suggest the existence of selective sensitivity in detecting abnormalities of spontaneous brain activity in CPI patients in slow-5 and slow-4 bands. These finding further suggest that different frequency bands might have specific pathological significances. Therefore, in addition to gender (Dai et al., 2016), we should also consider the frequency bands when measuring the intrinsic brain activity of CPI patients.

4.4. Correlations between sleep quality and abnormal ALFF in the slow-5 band

Our correlative analyses showed that the ALFF of the right PoCG/IPL in the slow-5 band was related to lower sleep quality (PSQI) and higher anxiety (STAI-s). An increased ALFF in the above-mentioned regions may reflect a hyperarousal state in sensory processing and prolong the sleep latency of CPI patients. Worsening sleep quality is often comorbid with emotional disorders (Ohayon and Roth, 2003), and hyperactivity in the PoCG/IPL may represent an important factor in anxiety associated with insomnia.

4.5. Limitations

The present study served as an exploratory study reporting on a universality alteration in patients with CPI. Several limitations should be considered when interpreting the results. First, the study had a small sample size. Second, caution should be used in the interpretation of the ALFF-neuropsychological correlation because these results did not survive the correction of multiple comparisons. Third, with regard to the reported frequency-dependent results, we mainly focused on the slow-5 and slow-4 bands, whereas other bands (slow-6, slow-3, and slow-2) were generally regarded as physiological noise and therefore discarded. However, high-frequency (>0.1 Hz) specific abnormalities have recently been identified in several disease states, including chronic somatic pain (Malinen et al., 2010), visceral pain (Hong et al., 2013), and late-onset depression (Yue et al., 2015), which suggests that the high-frequency information present in the rs-fMRI signal may provide useful information in clinical studies. Finally, we did not objectively assess the sleep quality or daytime sleepiness.

5. Conclusion

In this study, we provide evidence that CPI patients have abnormal ALFF values, including decreased ALFF in the several DMN sub-regions, the prefrontal cortex, integrated regions, and the right and left CPL, indicating a local dysfunction; increased ALFF was also observed in several posterior regions of the DMN, the secondary visual cortex and the somatosensory cortex. Specifically, we demonstrate that the ALFF changes in CPI patients exhibit different spatial patterns in different frequency bands. Abnormal ALFF was related to poor sleep quality (PSQI) and high anxiety (STAI-s) only in the slow-5 band. These findings suggest that abnormal sensory input and intrinsic information processing could be a hyperarousal mechanism in CPI patients. Furthermore, the frequency factor should be taken into consideration in exploring ALFF-related clinical manifestations.

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Conflict of interests

The authors declare that there are no conflicts of interest regarding the publication of this article.

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