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# Research report

# Frequency-dependent alterations in the amplitude of low-frequency fluctuations in social anxiety disorder



Youxue Zhang<sup>a,1</sup>, Chunyan Zhu<sup>b,1</sup>, Heng Chen<sup>a</sup>, Xujun Duan<sup>a</sup>, Fengmei Lu<sup>a</sup>, Meiling Li<sup>a</sup>, Feng Liu<sup>a</sup>, Xujing Ma<sup>a</sup>, Yifeng Wang<sup>a</sup>, Ling Zeng<sup>a</sup>, Wei Zhang<sup>b,\*</sup>, Huafu Chen<sup>a,\*\*</sup>

<sup>a</sup> Key Laboratory for NeuroInformation of Ministry of Education, School of Life Science and Technology,

University of Electronic Science and Technology of China, Chengdu 611054, Sichuan, PR China

<sup>b</sup> Department of Psychiatry, West China School of Medicine, West China Hospital of Sichuan University, Chengdu 610041, PR China

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#### ABSTRACT

*Background:* Recent studies on resting-state functional magnetic resonance imaging (fMRI) have found an abnormal temporal correlation between low-frequency oscillations (LFO) in social anxiety disorder (SAD). However, alterations in the amplitudes of these LFO remain unclear.

*Methods*: This study included 20 SAD patients and 20 age-, gender-, and education-matched healthy controls. Resting-state fMRI data were acquired using a gradient-echo echo-planar imaging sequence, and the amplitudes of LFO were investigated using the amplitude of low-frequency fluctuation (ALFF) approach. Two frequency bands (slow-5: 0.01–0.027 Hz; slow-4: 0.027–0.073 Hz) were analyzed.

*Results:* Significant differences in ALFF were observed between the two bands in widespread regions including the postcentral gyrus, precentral gyrus, medial prefrontal cortex (MPFC), orbitofrontal cortex, hippocampus, thalamus, caudate, putamen, and insula. Compared with the healthy controls, the SAD patients showed lower ALFF in the dorsolateral prefrontal cortex (DLPFC), MPFC, superior temporal gyrus, and insula but higher ALFF in the middle occipital gyrus. Furthermore, we found that the SAD patients had reduced ALFF in the MPFC in the slow-5 band.

Limitation: The small sample size may decrease the statistical power of the results.

*Conclusions:* SAD patients had frequency-dependent alteration in intrinsic brain activity. This finding may provide insights into the understanding of the pathophysiology of SAD.

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# 1. Introduction

Social anxiety disorder (SAD) or social phobia is a common and pervasive mental disorder characterized by excessive fear and avoidance of different social situations (Stein and Stein, 2008). Epidemiological research has revealed that the lifetime prevalence of SAD ranges between 4% and 16% (Ohayon and Schatzberg, 2010). Individuals with SAD typically fear and avoid the scrutiny of others, leading to significant functional impairment. However, the pathophysiological mechanism underlying SAD remains largely unclear.

Resting-state functional magnetic resonance imaging (fMRI) has recently been suggested as an important avenue to explore the

chenhf@uestc.edu.cn (H. Chen).

pathophysiological mechanisms underlying psychiatric and neurological diseases (Barkhof et al., 2014; Guo et al., 2011; Liu et al., 2012; Zhang et al., 2012). In contrast to conventional task-based fMRI, resting-state fMRI is easier to implement and requires minimal patient compliance; thus, using resting-state fMRI avoids potential performance confounders related to the task paradigms in clinical studies. Resting-state functional connectivity, defined as the temporal correlation of a neurophysiological index measured in different brain areas in the resting state, is a promising way to detect biomarkers in neuropsychiatric disorders (Greicius, 2008; Guo et al., 2013; Liu et al., 2014a). Using resting-state functional connectivity, scholars have found abnormal functional couplings in the default mode network (Liu et al., 2013a; Liu et al., 2014b; Zhao et al., 2007), the fronto-amygdala circuit (Hahn et al., 2011), and the dorsal attention network (Liao et al., 2010). These data indicate that SAD is associated with disrupted functional brain networks. However, these findings are based on investigations of low-frequency oscillations (LFO) from the perspective of temporal synchronization (functional connectivity) and not regional activity. Although aberrant functional connectivity between two regions is found in SAD, no conclusion can be drawn about which region is

<sup>\*</sup> Corresponding author. Fax: +862885582944.

<sup>\*\*</sup> Corresponding author at: Key laboratory for Neuro Information of Ministry of Education, School of Life Science and Technology, University of Electronic Science and Technology of China, Chengdu, Sichuan 610054, PR China. Fax: +862883208238.

E-mail addresses: weizhang27@163.com (W. Zhang),

<sup>&</sup>lt;sup>1</sup> Youxue Zhang and Chunyan Zhu contributed equally to this work.

abnormal. Thus, other methods are needed to characterize regional signal dynamics in SAD.

The amplitude of low-frequency fluctuation (ALFF) of the blood-oxygenation level-dependent approach is effective and powerful for examining disease-related local brain activity (Zang et al., 2007). Previous studies have observed abnormal ALFF in neuropsychiatric diseases, such as attention-deficit/hyperactivity disorder (Zang et al., 2007), major depressive disorder (Liu et al., 2013b), Alzheimer's disease (He et al., 2007), and schizophrenia (Guo et al., 2014). However, these studies typically utilized a low frequency band of 0.01–0.08 Hz; thus, their findings lack frequency specificity. LFO show different properties and physiological functions at different frequency bands (Buzsáki and Draguhn, 2004; Penttonen and Buzsáki, 2003). Recently, Zuo et al. (Zuo et al., 2010) have revealed that ALFF has distinct patterns in different low frequency bands and is more robust in the basal ganglia at the frequency band of 0.027-0.073 Hz. To date, ALFF has not been used to examine regional activity in SAD at different frequency bands.

Motivated by previous work, the present study employed ALFF to examine regional spontaneous neural activity at different frequency bands in SAD patients. On the basis of the previous findings, we hypothesized that SAD patients exhibited significant frequencydependent changes in ALFF compared with healthy controls.

## 2. Materials and methods

# 2.1. Participants

We recruited 20 SAD patients from the Mental Health Center of the Huaxi Hospital, Chengdu, China. None of the SAD participants received psychiatric and psychotherapy medications. The SAD diagnosis was identified by two attending psychiatrists and a trained interviewer using the Structured Clinical Interview DSM-IV (SCID)-Patients Version. The SAD patients with any history of major physical illness, neurological disease, cardiovascular disease, or a lifetime of drug use or alcohol were excluded from this study. We also recruited and screened 20 age-, sex-, and education-matched healthy controls using the SCID Non-Patients Version. The detailed demographic and clinical characteristics of the two groups are shown in Table 1. All participants completed the Liebowitz Social Anxiety Scale (LSAS), the Spielberger State-Trait Anxiety Inventory (STAI), the Hamilton

	SAD ( <i>n</i> =20)	HC (n=19)	SAD vs. HC	
	$\text{Mean}\pm\text{SD}$	$\text{Mean}\pm\text{SD}$	T value	p Value
Gender (male/female) Age (years)	14M/6F 22.09 + 3.99	14M/5F 21.89 + 3.77	- 0.91	0.801ª 0.369
Education (years)	$14.10 \pm 1.48$	$14.11 \pm 2.00$	0.01	0.993
Duration (months)	$45.40 \pm 39.78$	-	-	-
HAMD	$7.50 \pm 6.27$	$1.05\pm1.54$	4.36	< 0.001
HAMA	$7.50 \pm 6.27$	$0.89 \pm 1.52$	4.65	< 0.001
STAI-T	$48.25 \pm 7.02$	$32.58 \pm 4.85$	8.07	< 0.001
STAI-S (pre-scanning)	$41.35 \pm 8.31$	$31.05\pm4.72$	4.73	< 0.001
STAI-S (post-scanning)	$37.65 \pm 9.54$	$32.68 \pm 7.02$	1.84	0.073
LSAS (total score)	$53.90 \pm 11.50$	$19.21 \pm 7.68$	11.02	< 0.001
LSAS (fear factor)	$28.00 \pm 6.17$	$8.42 \pm 4.94$	10.90	< 0.001
LSAS (avoidance factor)	$25.90 \pm 6.93$	$10.79 \pm 4.79$	7.88	< 0.001
Mean FD	$\textbf{0.08} \pm \textbf{0.03}$	$0.09 \pm 0.04$	-0.93	0.35

SAD, social anxiety disorder; HC, healthy controls; LSAS, Liebowitz Social Anxiety Scale; HAMA, Hamilton Anxiety Rating Scale; HAMD, Hamilton Depression Rating Scale; STAI, State-Trait Anxiety Inventory; SD, standard deviation; FD, frame-wise displacement.

<sup>a</sup> The p Value was obtained by the Kruskal–Wallis test. The other p Values were obtained by the two-sample two-tailed t-test.

Anxiety Rating Scale (HAMA), and the Hamilton Depression Rating Scale (HAMD). The STAI questionnaires consist of two components: STAI-State (STAI-S) and STAI-Trait (STAI-T) scores. The former component evaluates the degree of state anxiety of the participants, whereas the latter component tests the inherent trait anxiety degree of the participants. The STAI-T questionnaire was completed immediately before and after the MRI scanning. This study was approved by the Ethics Committee of Huaxi Hospital, and each participant provided a written informed consent.

# 2.2. Image acquisition

Functional images were acquired on a 3.0-T GE-Signa MRI scanner in Huaxi hospital using a single-shot, gradient-recalled echo-planar imaging (EPI) sequence with the following settings: TR=2000 ms, TE=30 ms, flip angle=90°, field of view=24 cm, inplane matrix= $64 \times 64$ , slice thickness=5 mm, and voxel size= $3.75 \times 3.75 \times 5$  mm<sup>3</sup>. Thirty slices were collected with interleaved acquisition with no gap between slices. For each participant, 205 volumes were acquired, resulting in a total scan time of 410 s. Foam padding was used to minimize head motion, and the participants were instructed to rest with their eyes closed, not to think of anything in particular, and not to fall asleep.

## 2.3. Data preprocessing

Data preprocessing was carried out using SPM8 software (http://www.fil.ion.ucl.ac.uk/spm). The first five volumes were discarded to ensure steady-state longitudinal magnetization. Subsequently, we performed slice timing correction and head motion correction on the remaining 200 volumes. One of the healthy controls was excluded from further analysis because the head movement exceeded +1.5 mm in translation or  $+1.5^{\circ}$  in rotation. The mean frame-wise displacement (FD) was calculated to further determine the comparability of head movement across groups as suggested by Power et al. (2012). The resulting images were normalized to the standard EPI template and resampled to 3 mm cubic voxels. Then, the normalized images were smoothed with an isotropic Gaussian kernel (FWHM=8 mm), and linear trend removal was performed to reduce the effect of low-frequency drifts. We focused on LFO (typically defined as frequencies of 0.01-0.08 Hz) in this study. Data were filtered using the slow-5 band (0.01-0.027 Hz) and slow-4 band (0.027-0.073 Hz), which were consistent with recent studies (Han et al., 2011; Hou et al., 2014).

### 2.4. ALFF calculation

ALFF analysis was conducted by using REST software (Song et al., 2011). ALFF was calculated as previously described (Guo et al., 2012). Briefly, the time series were transformed to the frequency domain using a fast Fourier transform, and the power spectrum was obtained. The square root of the power spectrum was calculated and then averaged across the above-mentioned predefined frequency interval (i.e., slow-5 and slow-4 bands). This averaged square root was taken as the ALFF (Zang et al., 2007). For standardization purposes, we divided the ALFF value of each voxel by the global mean value in each participant.

# 2.5. Second-level analysis

To examine the effects of group and frequency band in ALFF, a two-way repeated-measures analysis of variance (ANOVA) was used. Group (the SAD patients vs. the healthy controls) served as a between-subject factor; frequency band (the slow-5 vs. the slow-4) as a repeated-measures factor; and gender, age, and mean FD as covariates. All the statistical maps were corrected for multiple comparisons to a significant level of p < 0.05 (AlphaSim corrected). This correction was determined by Monte Carlo simulations (Ledberg et al., 1998) using REST software (Song et al., 2011). Finally, post-hoc two-sample *t* tests were performed in the clusters that exhibit significant main effects and interaction between group and frequency band (p < 0.05, FDR corrected).

# 2.6. Correlation analysis between the abnormal ALFF and clinical severity

Brain regions showing significant differences between the SAD and healthy controls were extracted as regions of interest. To identify the relationships of the ALFF value in the regions with significant differences and clinical severities, linear correlation analyses were conducted between the mean ALFF value extracted from the significant clusters and the LSAS (including total score, fear factor, and avoidance factor) score. The threshold of p < 0.05 was considered to be significant.

# 3. Results

# 3.1. Main effect of the frequency band factor

The main effect of the frequency band from the two-way repeated-measures ANOVA is shown in Fig. 1 and Table 2. Com-

pared with the slow-5 band, the slow-4 band exhibited significantly higher ALFF in the postcentral gyrus, bilateral thalamus, bilateral caudate, bilateral putamen, and bilateral insula but significantly lower ALFF in the bilateral superior medial frontal gyrus, bilateral superior orbitofrontal gyrus, bilateral middle orbitofrontal gyrus, and bilateral superior temporal gyrus (p < 0.05, AlphaSim corrected).

# 3.2. Main effect of the group factor

The main effect of the group factor is shown in Fig. 2 and Table 3. Compared with the healthy controls, the SAD patients showed significantly higher ALFF in the bilateral middle occipital gyrus but lower ALFF in the bilateral dorsolateral prefrontal gyrus (DLPFC), bilateral medial prefrontal gyrus (MPFC), right superior temporal gyrus, and right insula (p < 0.05, AlphaSim corrected).

# 3.3. Interaction effects between the group and frequency band

Significant interaction was observed between the group and the frequency band. Further a post-hoc *t* test revealed that ALFF significantly decreased in the left MPFC in the slow-5 band in the SAD patients but did not significantly change in the slow-4 band (p < 0.05, FDR corrected) (Fig. 3, Table 4).



**Fig. 1.** Main effect of the frequency band factor on ALFF. Red color represents the higher ALFF and blue color represents the lower ALFF in the slow-4 band than slow-5 band. The results were obtained by a two-way repeated-measure ANOVA. AlphaSim corrected p < 0.05 (individual voxel threshold p < 0.05 and a minimum cluster size of 538 voxels).(For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

#### Table 2

Main effect of frequency band on the ALFF revealed by a two-way repeated-measures ANOVA.

	Brain regions	BA	Cluster size	F value	MNI coordinates		
					X	Y	Ζ
Slow-4 > slow-5							
Cluster 1	Bilateral insula Bilateral hippocampus Bilateral caudate Bilateral putamen Bilateral thalamus Left postcentral gyrus Left precentral gyrus	6/13/19	1215	174.2	39	- 39	3
Cluster 2	Right postcentral gyrus Right precentral gyrus	6/9	1275	317.2	27	15	24
Slow-5 > Slow-4							
Cluster 1	Bilateral superior orbitofrontal gyrus Bilateral middle orbitofrontal gyrus Bilateral superior medial frontal gyrus Bilateral superior frontal gyrus Bilateral superior temporal gyrus	9/10/21	1452	87	12	54	-21

BA, Brodmann's area; MNI, Montreal Neurological Institute. *X*, *Y*, *Z*, coordinates of primary peak locations in the MNI space. *F* statistical value of peak voxel showing ALFF differences. All the clusters survived p < 0.05, Alphasim corrected, a combined threshold of p < 0.05, and a minimum cluster size of 538 voxels. Degree of freedom=(1, 72).



**Fig. 2.** Main effect of the group factor on ALFF. Red color represents the higher ALFF and blue color represents the lower ALFF in the SAD group than healthy control group. The results were obtained by a two-way repeated-measures ANOVA. AlphaSim corrected p < 0.05 (individual voxel threshold p < 0.05 and a minimum cluster size of 203 voxels). SAD: social anxiety disorder; HC: healthy control.(For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

#### Table 3

Main effect of group on the ALFF revealed by a two-way repeated-measures ANOVA.

	Brain regions	BA	Cluster size	F value	MNI coordinates		
					X	Y	Ζ
SAD > HC Cluster 1 Cluster 2 HC > SAD	Right middle occipital gyrus Left middle occipital gyrus	19 19	432 321	13.09 19.59	36 36	-90 -84	18 15
Cluster 1	Right superior temporal gyrus Right insula	21/13	445	13.76	66	- 12	-9
Cluster 2	Bilateral DLPFC Bilateral MPFC	8/9	535	16.74	-24	48	30

SAD, social anxiety disorder; HC, healthy controls; DLPFC, dorsolateral prefrontal gyrus; MPFC, medial prefrontal gyrus; BA, Brodmann's area; MNI, Montreal Neurological Institute. *X*, *Y*, *Z*, coordinates of primary peak locations in the MNI space. *F* statistical value of peak voxel showing ALFF differences. All the clusters survived p < 0.05, Alphasim corrected, a combined threshold of p < 0.05, and a minimum cluster size of 203 voxels. Degree of freedom=(1, 72).



**Fig. 3.** The interaction between the frequency band and group on ALFF. Blue color represents the lower ALFF in the patients with SAD in the slow-5 band. The results were obtained by a two-way repeated-measures ANOVA and a post-hoc test. We found that decreased ALFF in the left MPFC in the patients with SAD in the slow-5 band. There were no significant differences in ALFF between the groups in the slow-4 band. FDR corrected p < 0.05. These findings reflect the results of the interaction of group and frequency band. SAD: social anxiety disorder; HC: healthy control.(For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

# 3.4. Correlation analysis between the abnormal ALFF and clinical severity

No correlation was found between the mean ALFF values in the aforementioned regions and the LSAS scores.

#### 4. Discussion

To the best of our knowledge, this is the first study to investigate the alterations in LFO amplitude in SAD patients at two frequency bands (the slow-5 and slow-4 bands). Several regions exhibited significant differences in ALFF between two bands and between two groups. A significant frequency bandgroup interaction was also observed in brain areas. Our findings demonstrated that the abnormal spontaneous neural activity in SAD patients was frequency dependent.

# 4.1. Differences in ALFF between frequency bands

Compared with the slow-5 band, the slow-4 band showed higher ALFF in the postcentral gyrus and precentral gyrus, particularly in the subcortical regions, including the thalamus, caudate, putamen, and insula. Increasing evidence in recent years has shown that the slow-4 band exhibits stronger ALFF than the slow-5 band in the subcortical regions (Han et al., 2011; Zuo et al., 2010). Furthermore, we also found higher ALFF in the orbitofrontal cortex, medial frontal gyrus, and superior temporal gyrus in the slow-5 band. These findings were consistent with previous reports that the slow-5 band has increased ALFF in the frontal cortex and default-mode regions (Wei et al., 2014; Zuo et al., 2010). These studies indicated that specific frequency bands are generated by different mechanisms with different physiological functions (Biswal et al., 1995).

### 4.2. Differences in ALFF between groups

Results of group difference showed that the SAD patients exhibited lower ALFF in the MPFC, DLPFC, insula, and superior temporal gyrus and higher ALFF in the middle occipital gyrus when compared with the healthy controls. Cognitive models of SAD suggest that this disorder is characterized by emotional biases

#### Table 4

Interaction between group and frequency band revealed by a two-way repeatedmeasures ANOVA and a post-hoc test.

Brain regions	BA	Cluster size	T value	MNI	MNI coordinates		
				X	Y	Ζ	
In the slow-5 band <b>SAD</b> < <b>HC</b> Left MPFC <b>SAD</b> > <b>HC</b> None In the slow-4 band None	8/9	128	- 3.85	-3	42	42	

SAD, social anxiety disorder; HC, healthy controls; MPFC, medial prefrontal gyrus; BA, Brodmann's area; MNI, Montreal Neurological Institute. *X*, *Y*, *Z*, coordinates of primary peak locations in the MNI space. *T* statistical value of peak voxel showing ALFF differences. All the clusters survived p < 0.05, FDR corrected. Degree of freedom=34.

and distorted negative self-beliefs (Clark and McManus, 2002; Clark and Wells, 1995). Failed emotion regulation is another critical feature of SAD (Goldin et al., 2009b). The DLPFC, as a pivotal region of the cognitive control network, plays an important role in emotion regulation (Phillips et al., 2008). DLPFC activation is decreased during emotion-related tasks (Gentili et al., 2008; Goldin et al., 2009a). These findings may provide novel insights into the pathophysiological mechanism of SAD.

Numerous studies have reported that MPFC is related to selfreferential processes and self-awareness (D'Argembeau et al., 2007; Johnson et al., 2002; Northoff and Bermpohl, 2004; Northoff et al., 2006). Impaired recruitment of the MPFC at the early stage of cognitive reappraisal highlights the dysfunction of emotion regulation in SAD patients (Goldin et al., 2009a). Moreover, this region is increasingly considered as a central region for storing and reasoning about the perspectives and beliefs of others (Fletcher et al., 1995; Gallagher et al., 2000; Mitchell et al., 2006). Our results agreed with previous studies and suggested that the dysfunction of emotion regulation in SAD patients is affected by abnormal activities in the MPFC.

Previous studies demonstrated insula hyperactivity in anxious individuals during negative emotion processing (Etkin et al., 2011; Etkin and Wager, 2007; Gentili et al., 2008). However, several pioneering resting-state fMRI studies observed decreased connectivity in the insula-cingulate network in generalized anxiety disorder and SAD patients (Etkin et al., 2009; Liao et al., 2010). Boshuisen et al. (2002) also reported that individuals with panic disorder exhibit hypoactivity in the insula under anticipatory anxiety conditions. Overall, the abnormal insula activities in SAD patients remain inconsistent and should thus be further investigated.

Decreased activity in the superior temporal gyrus is found when SAD patients react to negative self-beliefs (Goldin et al., 2009a). Essentially, SAD is characterized by making excessive negative and distorted predictions about social events, which may enhance perceptions of threat and cause excessive anxiety in social situations (Campbell-Sills and Barlow, 2007; Goldin et al., 2009a; Stein and Stein, 2008). The fundamental reason behind this social fear remains unclear, but it may be due to deficits in social cognition. Regions including the orbitofrontal cortex (Beer et al., 2006), prefrontal cortex (Wood, 2003), superior temporal gyrus (Adolphs, 2002), fusiform face area (Adolphs, 2002), and insula (Ebisch et al., 2011) comprise a network involved in socioemotional information processing. Cognitive and emotional information processing is deficient in SAD patients (Goldin et al., 2009a; Goldin et al., 2009b). Thus, these abnormal regions may provide the evidence for the dysfunction of socio-emotional information processing in SAD patients.

The occipital gyrus, which is located beside the fusiform gyrus, shows increased activity when SAD patients react to emotional facial expressions (Pujol et al., 2009; Straube et al., 2004). Qiu et al. (2011) documented that SAD patients have abnormal regional homogeneity (ReHo) in the left occipital gyrus relative to healthy controls. In addition, SAD patients have biased reaction to threatening or critical facial expressions (Coles and Heimberg, 2005; Foa et al., 2000). Therefore, we speculated that the increased ALFF in the occipital cortex is associated with the abnormal characteristic of social communication in SAD (Bögels and Mansell, 2004; Mogg et al., 1997).

# 4.3. Frequency-dependent changes in ALFF in SAD patients

In the present study, the ALFF in the SAD patients decreased in the MPFC in the slow-5 band but did not significantly change in the slow-4 band. This result reflected that the pattern of intrinsic brain activity was sensitive to specific frequency bands and that the slow-5 band might be sensitive in detecting abnormalities of spontaneous brain activity in the MPFC in SAD patients. Previous studies observed specific amplitude changes in resting-state brain fluctuations at different frequency bands in other diseases and demonstrated that the pattern of intrinsic brain activity is sensitive to specific frequency bands (Li et al., 2014). For example, Han et al. (2011) reported that ALFF decreases in the slow-5 band but does not change in the slow-4 band in amnestic mild cognitive impairment patients. Li et al. (2014) suggested that abnormal LFO amplitudes in the slow-5 band are specific and useful for subcortical ischemic vascular disease diagnosis. Moreover, schizophrenic patients exhibit widespread abnormalities in LFO amplitudes in the slow-4 frequency band (Hoptman et al., 2010), and in-depth diagnostic information is observed for children with attention deficit hyperactivity disorder in the slow-4 band (Di Martino et al., 2008). Although the physiological origin and specific functions of slow-4 and slow-5 frequency bands remain largely unclear, a prior research demonstrated that LFO allow for an integration of neuronal effects with large areas of involvement whereas high frequency oscillations are limited to small neuronal space (Buzsáki and Draguhn, 2004). Thus, our results implied that the slow-5 band might have greater sensitivity in detecting abnormalities of spontaneous brain activity in SAD patients than the slow-4 band. These findings further suggest that different frequency bands might have specific pathological significances. Therefore, future studies should investigate whether or not such frequency specific fluctuations could be used to diagnose SAD.

#### 4.4. Correlation analysis

No correlation was found between the ALFF value of abnormal brain regions in the SAD patients and the LSAS scores. Therefore, altered ALFF cannot be regarded as a quantitative marker to evaluate clinical symptom severity in SAD patients, although it can be applied to reveal functional aberrant brain regions. Selffocus, emotional biases, and negative self-beliefs, which are not well investigated in the LSAS, might be associated with abnormal ALFF. These factors warrant further exploration in future studies.

#### 5. Limitation

Several limitations should be noted in this study. First, our sample size was relatively small. Thus, the findings in the present study should be replicated in a large clinical sample size in the future. Second, a relatively low-sampling rate (TR=2 s) was used

for multislice acquisitions. Under this sampling rate, inevitable physiological noises such as respiratory and heart-beat fluctuations were reduced but could not be entirely removed. A rigorous method should be applied to remove such physiological noises in the future. Third, the main effects of band and group were the majority of the results and the interaction only appeared in the MPFC, which might mean that the mass of other group differences appeared not to be dependent on the frequency band. However, we found that some regions had trends toward significant differences. To some extent, this might be due to the small sample size used in this study. Future study will use large sample size to increase the statistical power. Finally, previous studies divided the whole frequency band and analyzed all sub-bands (Baliki et al., 2011; Baria et al., 2011). Considering that the current study only focused on the LFO (typically defined as frequencies of 0.01-0.08 Hz), we merely investigated the between-group ALFF changes in the slow-5 and slow-4 frequency bands. The obtained results were consistent with other similar studies (Han et al., 2011; Hou et al., 2014). Future investigations on the ALFF changes in all frequency sub-bands are warranted.

#### 6. Conclusion

This resting-state fMRI study provided evidence on the abnormal LFO amplitude in many brain regions of the SAD patients. The changes in ALFF between the SAD and healthy controls were modulated by the frequency band. The significant interaction identified in the MPFC showed that specific frequency bands were involved with different physiological functions. These results demonstrated that the abnormalities of LFO amplitude in SAD patients were frequency dependent. This study provided novel insights into the pathophysiological mechanism of SAD.

#### **Conflict of interest**

No conflict of interest is declared.

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