1 Research report

Abnormal spontaneous neural activity in the anterior insula and anterior cingulate cortices in anxious depression

Q1 Chun-Hong Liu [a,b], Xin Ma [a,b,*], Lu-Ping Song [c], Jin Fan [d], Wei-Dong Wang [e], Xue-Yu Lv [e], Yu Zhang [a,b], Feng Li [a,b], Lihong Wang [f,**], Chuan-Yue Wang [a,b]

[a] Beijing Key Laboratory of Mental Disorders, Department of Psychiatry, Beijing Anding Hospital, Capital Medical University, Beijing 100088, China
[b] Laboratory of Brain Disorders, Beijing Institute for Brain Disorders, Capital Medical University, Beijing 100069, China
[c] Rehabilitation College of Capital Medical University, China Rehabilitation Research Center, Beijing 100068, China
[d] Department of Psychology, Queens College, The City University of New York, Flushing, NY 11367, USA
[e] Department of Psychology, Guang'an Men Hospital, China Academy of Chinese Medical Sciences, Beijing 100053, China
[f] Center for Biomedical Imaging Research, Department of Biomedical Engineering, Tsinghua University, Beijing 10084, China

HIGHLIGHTS

- To examine adult anxious depression patients using ALFF and fALFF method.
- Increased ventral cingulate activity might be related to neurobiology of anxious depression.
- Increased insular activity might be related to the core symptoms of anxious depression.

ARTICLE INFO

ABSTRACT

Objective: Anxious depression is a distinct clinical subtype of major depressive disorder (MDD) characterized by palpitations, somatic complaints, altered interoceptive awareness, high risk of suicide, and poor response to pharmacotherapy. However, the neural mechanisms of anxious depression are still not well understood. In this study we investigated changes in neural oscillation during the resting-state of patients with anxious depression by measuring differences in the amplitude of low-frequency fluctuation (ALFF).
Methods: Resting-state functional magnetic resonance imaging was acquired in 31 patients with anxious depression, 18 patients with remitted depression, as well as 68 gender- and age-matched healthy participants. We compared the differences both in the ALFF and fractional ALFF (fALFF) among the three groups. We also examined the correlation between the ALFF/fALFF and the severity of anxiety as well as depression.
Results: Anxious depression patients showed increased ALFF/fALFF in the right dorsal anterior insular cortex and decreased ALFF/fALFF in the bilateral lingual gyrus relative to remitted depression patients and healthy controls. The increased ALFF in the dorsal anterior insula was also positively correlated with stronger anxiety in the anxious depression group. Anxious depression patients also displayed increased fALFF in the right ventral anterior cingulate cortex (ACC) compared to remitted depression patients and healthy controls.
Conclusions: Our results suggest that alterations of the cortico-limbic networks, including the right dorsal anterior insula and right ventral ACC, may play a critical role in the physiopathology of anxious depression.

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1. Introduction

Q2 Anxious depression is a common clinical subtype of major depressive disorder (MDD) characterized by dysphoric mood, disturbed sleep, somatic complaints, altered interoceptive awareness, and increased morbidity [1–3]. Compared with depression without Q3 anxiety, anxious depression has a greater severity of depressive

* Corresponding author at: Beijing Anding Hospital, Capital Medical University, No. 5 Ankang Lane, Dewai Avenue, Xicheng District, Beijing 100088, China. Tel.: +86 10 58303002; fax: +86 10 58303078.
** Corresponding author. Tel.: +86 10 62798401; fax: +86 10 62796175.
E-mail addresses: maxinanding@vip.163.com (X. Ma), lihong001@gmail.com (L. Wang).

illness, longer illness chronicity, and a higher risk of disability and suicidal tendency [4–6]. Anxious depression is also more likely to exhibit somatic symptoms, to take twice as long to recover from a depressive episode, and to have lower remission rates [7]. Despite the poor clinical outcomes and increasing social and economic burdens of anxious depression [8], little attention has been paid to the neurobiology of the disorder. There is a compelling need to investigate the underlying neural mechanisms of anxious depression to develop a better target for treatment.

The existing psychological models of anxious depression (such as the valence-arousal and approach-withdrawal models) are focused on anxiety-related hyperarousal. However, limited neuroimaging studies in literature have found widespread structural and functional changes in anxious depression within the cortico-limbic circuits including the anterior cingulate cortex (ACC), prefrontal cortex, middle temporal gyrus, and insula [2], which are largely overlapped with the regions involved in major depression. It is unclear about which structural or functional changes observed in the literature are specifically related to anxious depression and which to depression in general. The only functional MRI study that has compared depression with high versus low anxiety had a small sample size and studied only older adults [9]. This study revealed that elderly depressed subjects with high anxiety showed stronger functional connectivity in the posterior regions of the default mode network (e.g., the precuneus), and lower functional connectivity in the anterior regions of the default mode network (e.g., the rostral ACC, medial prefrontal cortex and orbitofrontal cortex) compared with low anxiety depressed subjects during resting-state using posterior cingulate cortex from automated anatomical labeling template as seed point. Although functional connectivity can disclose network changes related to anxious depression, it does not address which changes and regions are related to primary deficits in the disorder. Given the autonomic nervous deficits associated with anxious depression and the insula's role in interception [10], activity change in the insula might be associated with the anxiety-related symptoms of anxious depression [11,12]. To test this hypothesis, we examined the amplitude of low-frequency fluctuations (ALFF) and fractional ALFF (fALFF) during resting-state which allowed us to compute the strength of neural oscillation in each voxel instead of connectivities between regions.

The ALFF and fALFF are thought that can reflect the strength of intrinsic spontaneous neuronal activity [13,14]. ALFF measures the regional intensity of spontaneous fluctuations by integrated the square root of power spectrum in a low-frequency range [15] and fALFF is the ratio between the low frequency band and the entire detectable frequency range in a given signal without filtering [14]. ALFF reflects the absolute strength or intensity within a specific low frequency range, whereas fALFF represents the relative contribution of the low frequency band to the whole detectable frequency range in a given signal [16]. Abnormal ALFF and fALFF measurements have been found in a number of psychiatric disorders including Alzheimer's disease [17] and major depression [16,18]. Therefore, in this study, we used ALFF and fALFF to reveal neural alteration which is related to the pathology of anxious depression. In addition, in order to compare the changes in the ALFF and fALFF of anxious depression patients with those of healthy controls, we also included remitted depression patients to investigate the anxiety depression state effect. We also examined whether anxiety severity was specifically correlated with the changes in the ALFF and fALFF measurements. Our hypothesis was that anxious depression patients might have an altered ALFF or fALFF in the insula and cortico-limbic circuits.

2. Materials and methods

2.1. Participants

This study was approved by the Institution of Review Boards of Beijing Anding Hospital, Capital Medical University and State Key Laboratory of Cognitive Neuroscience and Learning, Beijing Normal University. Participants included 31 patients diagnosed with anxious depression, 18 patients diagnosed with remitted depression and 69 healthy controls. All participants were right-handed, determined by the Edinburgh Inventory of handedness [19]. The criteria of selection for patients were as follows (also described in [18]): (1) between the ages of 18 and 60 years and has the ability to give voluntary informed consent; (2) meets the Structured Clinical Interview DSM-IV Axis I Disorders (SCID) diagnostic criteria for MDD; (3) no other psychiatric illnesses (e.g., schizophrenia, obsessive–compulsive disorder, and no alcohol or substance abuse or dependence) and no neurological illnesses; and (4) able to be scanned by MRI. The Hamilton Depression Rating Scale (HAMD) [20] was used to measure depressive symptoms on the day of scanning. Patients were grouped into anxious depression and remitted depression based on the anxiety/somatization factor score of the Hamilton Rating Scale for Anxiety (HAMA) and the HAMD. Anxious depression was defined as a total HAMA score of 15 or higher and a total HAMD score of 17 or higher, whereas the remitted depression was defined as a total HAMA score of eight or lower and a total HAMD score of eight or lower [9,21]. The healthy controls were recruited from the local community. The non-patient edition of the Structured Clinical Interview for the DSM-IV [22] was used to screen the healthy controls. Participants were excluded as healthy controls if they reported a history of neurological or neuropsychiatric disorders, or a positive family history of psychiatric disorders.

2.2. Image acquisition

Two hundred and forty contiguous gradient echo planar imaging (EPI) functional volumes were acquired with 33 axial slices, with parameters of repeat time (TR) = 2000 ms; echo time (TE) = 30 ms; flip angle (FA) = 90°; matrix size = 64 × 64; thickness/gap = 3.5/0.6 mm; and sequence duration = 480 s for each subject using a Siemens Trio 3.0 T scanner at the National Key Laboratory for Cognitive Neuroscience and Learning, Beijing Normal University, Beijing, China. One hundred and twenty-eight (128) slices of structural 3D-T1 weighted images were also acquired sagittally without gaps (TR = 2530 ms; TE = 3.39 ms; slice thickness = 1.33 mm; field of view (FOV) = 256 mm × 256 mm; in-plane resolution = 256 × 256; inversion time (TI) = 1100 ms; voxel dimension = 1 mm × 1 mm × 1.33 mm; and FA = 7°). All participants were instructed to close their eyes and relax but not to fall asleep.

2.3. Data preprocessing

EPI data was first preprocessed using Data Processing Assistant and Resting-State fMRI (DPARSF) [23] based on statistical parametric mapping 8 (SPM8, http://www.fil.ion.ucl.ac.uk/spm) using MATLAB R2009a (The Mathworks, Natick, MA). The first 10 volumes of functional time points were discarded to reach stability of initial MRI signal and allow the participants to adapt to the MRI acquisition environment. The remaining 230 scans were slice-timing correction and head motion correction. One healthy control was excluded from further analysis due to excessive head motion (head movements exceeded 2 mm in translation or 2° of rotation). Next, the motion corrected functional volumes were spatially normalized

using the standard EPI template and resampled to the voxel size of $3\,mm \times 3\,mm \times 3\,mm$. Subsequently, functional images were spatially smoothed with a $4 \times 4 \times 4$ full-width at half maximum (FWHM) kernel. Finally, the linear trend of the time series was detrended and band-pass filtering (0.01–0.08 Hz) was performed to remove the influence of low-frequency drift and high-frequency physiological noise.

2.4. ALFF and fALFF analysis

The filtered time course were converted to the frequency domain using fast Fourier transform (FFT) [24]:

$$x(t) = \sum_{k=1}^{N} (a_k \cos(2\pi f_k t) + b_k \sin(2\pi f_k t))$$

The ALFF [15] is the averaged squared root of the Fourier coefficient across 0.01–0.08 Hz at each voxel as:

$$\text{ALFF} = \sum_{k:f(k)\in[0.01,0.08]} \sqrt{\frac{a_k^2(f_k) + b_k^2(f_k)}{N}}$$

The fALFF [25] is the ratio of the amplitude (0.01–0.08 Hz) to that of the entire frequency range (0–0.25 Hz):

$$\text{fALFF} = \frac{\sum_{k:f(k)\in[0.01,0.08]} \sqrt{\frac{a_k^2(f_k) + b_k^2(f_k)}{N}}}{\sum_{k=1}^{N} \sqrt{\frac{a_k^2(f_k) + b_k^2(f_k)}{N}}}$$

The standardized ALFF and fALFF of each voxel was calculated by taking the degree of its raw ALFF or fALFF value and dividing it by the individual mean ALFF or fALFF value of the whole brain [16]. Finally, the smoothed standardized individual ALFF and fALFF maps were used for statistical analysis.

2.5. Statistical analysis

One-way analysis of variance (ANOVA) was performed to determine the ALFF and fALFF differences among the three groups (anxious depression patients, remitted depression patients, and healthy control subjects) using the statistical analysis panel implemented in the Resting State fMRI Data Analysis Toolkit (REST) (with age, gender and years of education as covariates) [26]. The corrected threshold was determined using the AlphaSim program [27], with the threshold set at $p < 0.01$ and a cluster size $>432\,mm^3$ (16 voxels, with a gray matter mask). As fALFF can effectively suppress the influence of motion and pulsatile effects and significantly improve the specificity and sensitivity of detecting spontaneous regional fluctuations [14,25,28], the aforementioned fALFF ANOVA results (rather than the ALFF ANOVA results) were used to identify the cerebral regions showing significant group differences as regions of interest (ROIs) [16,28,29]. Mean ALFF and fALFF values were extracted from each ROI for anxious depression subjects, remitted depression subjects, and healthy controls using REST. Since the HAMD and HAMA scores are highly correlated ($r = 0.912$, $p < 0.001$), we did not run the correlation separately for the HAMD scores. To investigate the relationship between the mean ALFF or fALFF values and a subject's clinical profile (such as disease duration, number of depressive episodes, and HAMA total scores), Pearson correlation coefficient analyses were performed on the pooled depression patients, including anxious depression and remitted depression patients with significant value at $p < 0.008$ (multiple comparisons correction). To examine psychotropic medication effects, all depression patients (including anxious depression and remitted depression) were assigned to receive antidepressant medication ($n = 42$) and not medicated ($n = 7$). Two sample t-tests
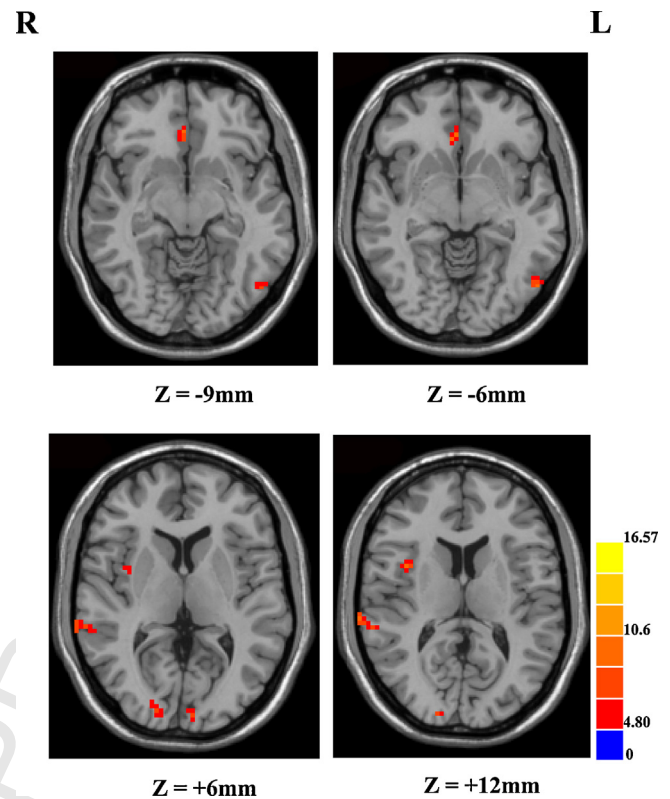
Fig. 1. Analysis of variance (ANOVA) differences in fractional amplitude of low-frequency fluctuations (fALFF) among the three groups. The left side of the figure corresponds to the right side of the brain.

were adopted to identify differences between depression patients on psychotropic medication, depression patients off psychotropic medication, and healthy controls.

3. Results

3.1. Demographic and clinical data

As shown in Table 1, there were no significant differences in the participants' ages or sex (all $p > 0.05$) among the three groups. However, there were significant differences in educational level among the three groups ($p < 0.05$), and significant differences between the HAMD and HAMA scores of the anxious depression and remitted depression groups. No significant difference was observed in duration of illness or number of depressive episodes between the anxious depression and remitted depression groups ($t = -0.35$, $p = 0.73$; $t = 0.48$, $p = 0.64$).

3.2. Group differences in fALFF and ALFF

The one-way ANOVA on fALFF revealed six clusters with significant differences across the three groups of participants, including right ventral ACC, right dorsal anterior insula, left middle temporal gyrus, right superior temporal gyrus, and bilateral lingual gyrus (Fig. 1 and Table 2). We also perform our analysis with one-way ANOVA on ALFF as used in Zang et al. [15]. As shown in Fig. 2 and Table 2, the results also showed cortico-limbic dysfunction, including the left middle temporal gyrus. Fig. 3 illustrates the results of the six significant clusters from the voxel-wised analysis and compared ALFF and fALFF among the three groups directly. Compared with healthy control subjects, anxious depression patients showed a significantly decreased ALFF and fALFF in the left middle temporal gyrus, bilateral lingual gyrus, and an increased ALFF and fALFF

Table 1
Group demographics and clinical measures.

Measure (mean ± SD)	Anxious depression patients ($n = 31$)	Temitted depression patients ($n = 18$)	Healthy controls ($n = 68$)	Value	p value
Age, years	36.35 ± 13.28	36.33 ± 12.12	35.55 ± 12.43	0.56	0.946#
Education level (years)	14.84 ± 3.10	15.00 ± 3.31	14.37 ± 3.21	0.406	0.667#
Sex (male/female)	14/17	8/10	34/34	0.299	0.861△
HAMD	22.61 ± 4.48	4.78 ± 2.88		15.122	0.00*
HAMA	22.94 ± 6.43	4.00 ± 2.4		14.716	0.00*
Duration of illness (years)	7.60 ± 9.44	8.50 ± 7.36		−0.349	0.729*
Number of depressive episodes	2.29 ± 1.74	2.06 ± 1.51		0.478	0.64*
Antidepressants	26	16			
SSRI	19	13			
SNRI	2	1			
Mitazapine	1	0			
Trazodone	2	0			
TCA	1	1			
Flupentixol and melitracen	1	1			
Antipsychotics	3	4			
Quetiapine	2	1			
Resperidone	1	2			
Aripiprazole	0	1			
Benzodiazepines	3	1			
Lonazepan	2	0			
Oxazepam	1	1			
Medication-free	5	2			

Abbreviations: SD: standard deviation; HAMD: Hamilton depression.
Rating scale. # indicates p values for one-way ANOVA and * indicates p values for two-sample t-tests. △ indicates p values for chi-square test.

in the right dorsal anterior insula, right superior temporal gyrus, and right ventral ACC. Anxious depression patients also displayed increased ALFF in the right dorsal anterior insula and decreased ALFF in the bilateral lingual gyrus relative to the remitted depression patients. However, when compared with remitted depression patients, anxious depression patients demonstrated significantly increased fALFF in the right ventral ACC, right dorsal anterior insula, and right superior temporal gyrus, and decreased fALFF in the left middle temporal gyrus and bilateral lingual gyrus. The remitted depression patients and healthy controls were not significantly different in the ALFF or fALFF in left middle temporal gyrus, bilateral lingual gyrus, the right dorsal anterior insula, right ventral ACC, or any other brain regions.

3.3. Psychotropic medication effects

There was no obvious difference between depression patients on psychotropic medication and those off psychotropic medication. Compared to the healthy controls, the depression patients who receive psychotropic medication showed decreased ALFF/fALFF in the left lingual gyrus and left middle temporal gyrus and increased ALFF/fALFF in the right ventral ACC and right dorsal anterior insula (Fig. 4).

3.4. Correlations between ALFF/fALFF values and clinical data

There was a significantly positive correlation in the ALFF and fALFF values of the right dorsal anterior insula and the HAMA score (ALFF, $r = 0.536$, $p < 0.001$; fALFF, $r = 0.519$, $p < 0.001$) in the pooled depression group (Fig. 5). We also found significantly positive correlations between regional fALFF values and the HAMA scores in the right ventral ACC in the pooled depression group ($r = 0.460$, $p = 0.001$) (Fig. 6). The detailed correlation results between the fALFF measurements and HAMA scores are presented in Table 3. No brain loci demonstrated significant correlations with the number of depressive episode or duration of illness.

4. Discussion

In this study, we used ALFF and fALFF measurements to examine whole-brain spontaneous activity in patients with anxious

Table 2
Brain regions showing ANOVA differences in the fALFF/ALFF values among the anxious depression, nonanxious depression, and healthy control groups.

Brain region	Side	BA	MNI coordinates			Voxels	F value
			x	y	z		
fALFF							
Ventral ACC	R	11.24	6	30	−6	16	8.58
Dorsal anterior insula	R		42	6	15	20	11.93
Middle temporal gyrus	L	37	−51	−69	0	45	12.50
Superior temporal gyrus	R		72	−30	12	30	9.80
Lingual gyrus	R		18	−93	6	16	8.45
Lingual gyrus	L	21	−15	−99	0	34	12.24
ALFF							
Middle temporal gyrus	L	18	−63	−36	−3	20	6.88
Middle occipital gyrus	R		33	−96	−6	40	9.13
Lingual gyrus	L		−12	−102	−3	44	10.11
Calcarine	L		−24	−63	9	16	8.48
Cuneus	R		12	−72	21	38	7.96

Abbreviations: ANOVA: One-way analysis of variance; fALFF: fractional amplitude of low-frequency fluctuation; BA: Brodmann area; MNI: Montreal Neurological Institute; ACC: anterior cingulate cortex; L = Left; R = Right.

R   L
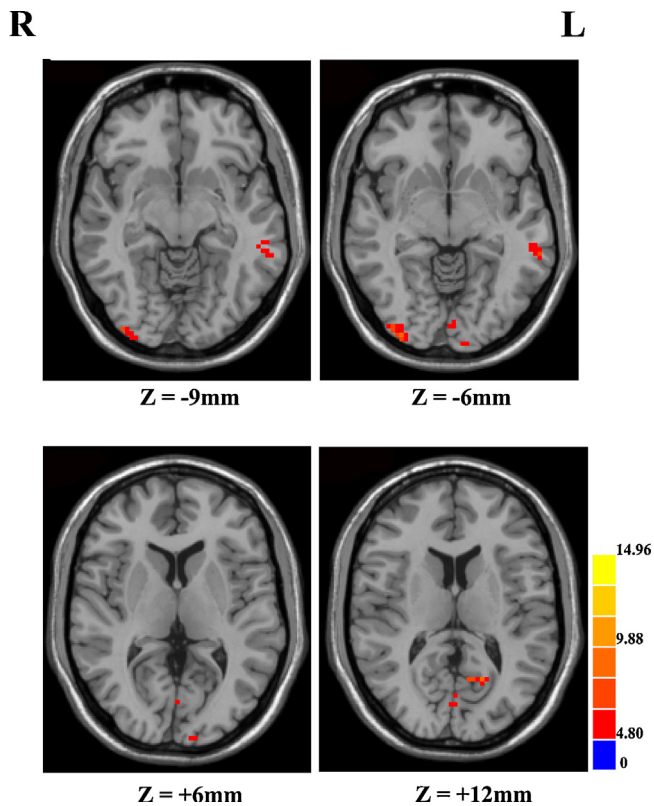
Fig. 2. ANOVA differences in the amplitude of low-frequency fluctuations (ALFF) values among the three groups. The left side of the figure corresponds to the right side of the brain.

depression. We found that patients with anxious depression had increased ALFF and fALFF values in the right dorsal anterior insula and decreased ALFF and fALFF values in the bilateral lingual gyrus relative to both, patients with remitted depression and healthy controls. The increased ALFF/fALFF values in the anterior insula positively correlated with severe anxiety symptoms in the pooled depression group. Moreover, patients with anxious depression displayed increased fALFF values in the right ventral ACC, which positively correlated with the HAMA scores. These findings supported our hypothesis that aberrant intrinsic neural fluctuations in patients with anxious depression are mainly located within corti-colimbic circuits, especially the insula and ventral ACC, which is important for automatic emotion regulation, decision making, and cognitive processes.

The anterior insula is an important cortical structure in the salience network, which is thought to detect internal and external stimuli and to initiate switches between self-referential processing in the default mode network and goal-directed, higher-level

Table 3
Regions which showed significantly correlation between their fALFF intensity and HAMA scores.

Brain regions	Side	r	p
Positive correlation			
Ventral anterior cingulate	Right	0.460	=0.001
Dorsal anterior insula	Right	0.519	<0.001
Superior temporal gyrus	Right	0.575	<0.001
Negative correlation			
Middle temporal gyrus	Left	−0.410	=0.001

Abbreviation: fALFF, fractional amplitude of low-frequency fluctuation; HAMA: Hamilton rating scale for anxiety.

cognitive effort in the central executive network [30,31]. Therefore, increased ALFF/fALFF values in the right anterior insula, as well as its significant correlation with the severity of anxiety, suggested an involvement of the right anterior insula in the pathology of the anxiety symptoms in anxious depression. Studies in literature also support our speculation. Baur et al. [32] reported that connectivity between the subregions of the insula and the amygdala is related to anxiety levels in healthy subjects. With proton magnetic resonance spectroscopy, Rosso et al. [12] found reduced levels of gamma-aminobutyric acid in the right anterior insular cortex in adults with posttraumatic stress disorder, which is associated with significantly higher state and trait anxiety. Avery et al. [33] reported decreased activity in the bilateral dorsal midinsular cortex during interoceptive attention tasks in unmedicated patients with depression relative to healthy controls. They also found that increased activity in the bilateral midinsula correlated with the severity of depression and the somatic symptoms. The findings in the above studies support those of the present study in suggesting that increased intrinsic neural oscillations in the anterior insula are a core characteristic of anxious depression.

Patients with anxious depression also demonstrated significantly increased fALFF values in the right ventral ACC relative to patients with remitted depression and healthy controls. The ventral ACC is an important region thought to be pivotal to the regulation of affect, visceromotor function, emotional processing, somatosensory processing, and self-referential processing [34]. There is abundant evidence in the literature of changes in this region in depression and anxiety disorders. For example, Drevets et al. [35] reported increased ventral ACC metabolism during depressed relative to remitted phases in the same subjects with major depression. Sheline et al. [36] reported a positive association between dorsal nexus (including a portion of the subgenual ACC) connectivity values and depression severity. Greicius et al. [37] also showed increased subgenual cingulate activity in depression. However, there are also seemingly contradictory findings. A study on panic disorder found reduced volume of the right ventral ACC [38]. Decreased subgenual ACC-precuneus connectivity in adolescent depression correlated with higher levels of depression severity [39]. However, decreased volume and decreased functional connectivity may not necessarily imply decreased regional activity. Importantly, the increased fALFF values in the ventral ACC were associated with the severity of anxiety, reflected by the HAMA scores in our study. Consistent with evidence from previous studies, increased resting state activity in the ventral ACC might also be a neuroimaging marker of anxious depression.

The middle temporal gyrus is activated in tasks involved in decision-making processes [40,41] and semantic memory [42,43]. The posterior portion of the superior temporal gyrus is a key node for the theory of mind [44]. We observed decreased fALFF values in the left middle temporal gyrus and increased values in the right superior temporal gyrus of patients with anxious depression relative to remitted patients and healthy controls. We speculate that functional deficits in the middle and superior temporal gyri, as well as the ventral ACC, may provide the neurobiological basis for aberrant emotional dysregulation and impaired decision making in anxious depression. Wang et al. [45] found decreased regional homogeneity in the left middle and right inferior temporal gyri in first-episode, drug-naïve patients with MDD. Lee et al. [46] reported that the gray matter concentration of the middle-superior temporal gyri was decreased in 47 patients with MDD. Carlson et al. [47] found improvements in depression ratings that correlated with metabolic changes in the right middle and superior temporal gyri with positron emission tomography following ketamine treatment. Based on previous findings, we speculate that low fALFF values in the middle and superior temporal gyri might be related
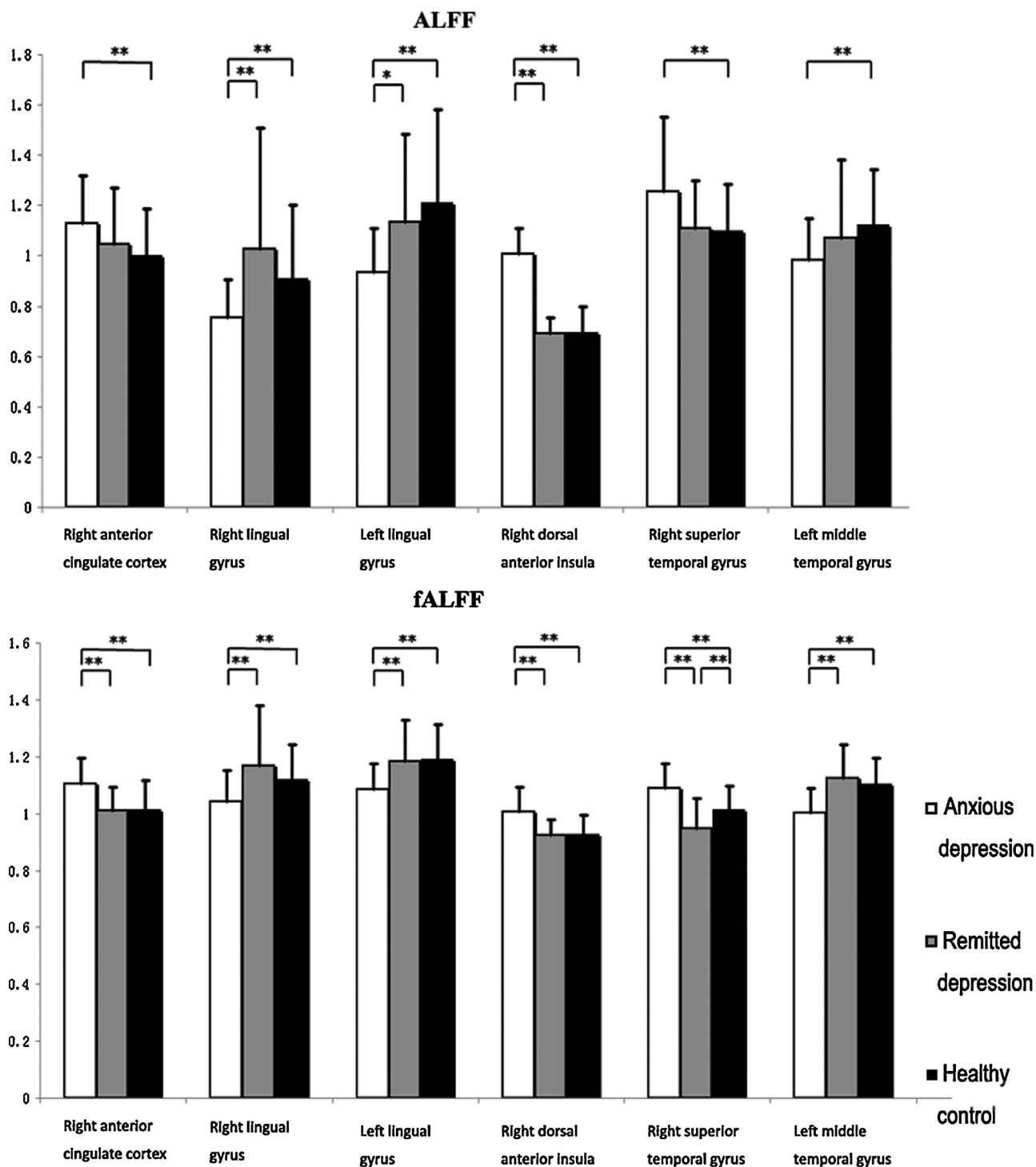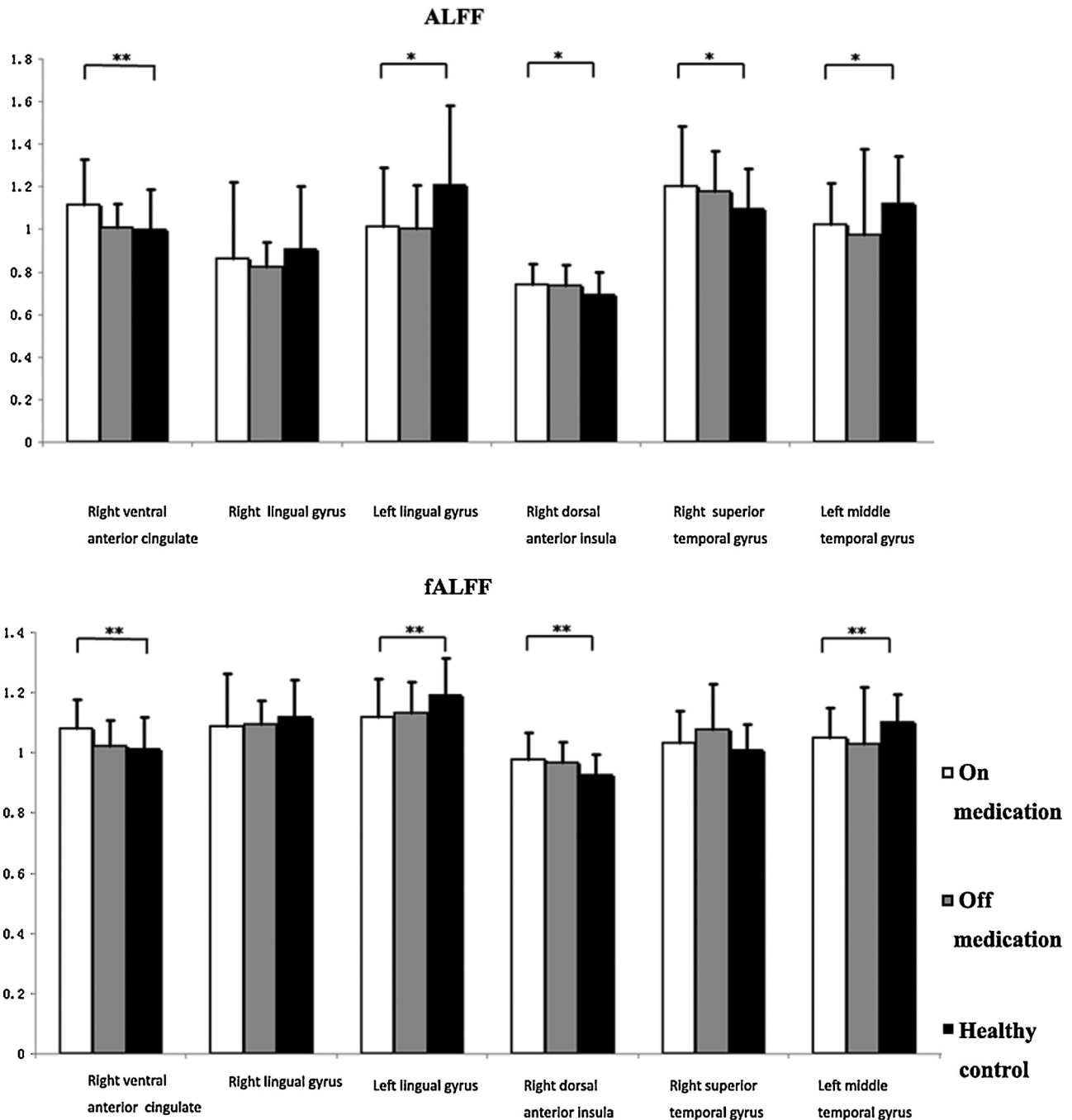
Fig. 3. Comparisons of the mean ALFF values (upper) and fALFF values (bottom) in each ROI across the anxious depression, remitted depression, and healthy control groups. The black Π-shaped lines indicate significant difference between two groups in the comparisons. The single asterisks represent a significance level of $0.01 < p < 0.05$, while the double asterisks indicate a significance level of $p < 0.01$.

to the dysfunction of emotional memory and social interactions in anxious depression.

In this study, we also found decreased ALFF and fALFF values in the bilateral lingual gyrus in the anxious depression group compared to the other two groups. Jing and colleagues (2013) found reduced ALFF/fALFF values in the left lingual gyrus in patients with current depression relative to healthy controls. The lingual gyrus is within the visual system, and links to the posterior insular, playing an important role in integrating visual information with introspective sensation or introspective stimuli [48,49]. Therefore, we speculate that the decreased ALFF and fALFF values in our study may indicate impairments in introspective integration processing in anxious depression. This is consistent with our previous study that showed decreased fALFF values in the lingual gyrus in the group with depression relative to a sibling group as well as a healthy control group [18]. The decreased ALFF and fALLF measurements in

Fig. 4. Comparisons of the mean ALFF or fALFF values in each selected region between patients with depression who were on psychotropic medication, patients with depression who were off psychotropic medication, and healthy controls. ** indicates $p \leq 0.01$; * indicates $0.01 < p \leq 0.05$.

our study, along with the evidence from previous studies, suggest that the impairments in introspective integration associated with anxious depression may also be a depressive state marker.

Functional connectivity analysis is a biased approach that lacks global and independent views due to its priori selection of the seed voxel or region [50]. In the present study, we used ALFF and fALFF to investigate the strength or intensity of low-frequency oscillations at each voxel; this was a data-driven unbiased analysis. A noticeable difference between our current findings and those reported by Andreescu et al. [9] was that, instead of focusing only on the default mode network, we investigated voxel-wise changes in the whole brain. Moreover, the fALFF is advantageous over ALFF in several aspects, such as higher sensitivity and specificity and fewer

biases from nonspecific physiological noise [14]. However, as the root mean square of low-frequency oscillations in the white matter is about 60% lower than that in gray matter, the ALFF measurement has higher test–retest reliability in gray matter [25,51]. In our results (Fig. 2), more regions showed significant group differences in the fALFF measurements than in the ALFF, possibly caused by the effective suppression of fALFF of the nonspecific signals, which significantly improved the specificity and sensitivity of regional brain spontaneous fluctuations.

There were several limitations in our study. First, almost all of the patients were on psychotropic medication due to ethical considerations. We also lacked detailed information regarding medication doses or the duration of treatment in the two patient
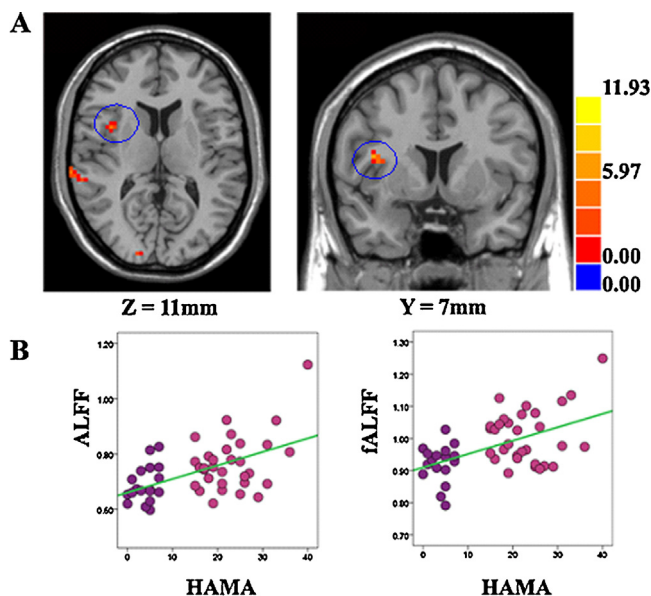
Fig. 5. (A) ANOVA differences in fALFF among the three groups. The color bar signifies the *F*-value of the group analysis. The degree of freedom for the *F* statistic in the right dorsal anterior insula is 11.93. (B) Pearson correlation analysis between the individual mean ALFF or fALFF value of the right dorsal anterior insular (peak coordinate: 42, 6, 15; cluster size: 20) and the HAMA scores in the pooled depression group (ALFF, $r = 0.536$, $p < 0.001$; fALFF, $r = 0.519$, $p < 0.001$). The left side of the figures indicates the right side of the brain. The dark-purple dots represent patients with remitted depression, and the light-purple dots represent patients with anxious depression. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

groups. Therefore, we cannot rule out the potential impact of medication. Psychotropic medications have been implicated in altered synaptic plasticity and neuroprotective, neurogenetic, and anti-inflammatory actions [45,52–54]. We found no obvious differences between patients with psychotropic medication-treated depression and those without psychotropic medication treatment. Second, the current study design cannot conclusively specify the role of the dorsal anterior insula in the neuropathology of anxious depression given the fact that the HAMD and HAMA scores correlated highly. Distinguishing the role of the insula in anxiety from that in depression, would require analysis of patients with active depression with high versus low anxiety. Future studies involving larger numbers of nonmedicated subjects with remitted and active depression are needed to exclude medication effects and to better

Fig. 6. (A) ANOVA differences in fALFF values among the three groups. The color bar signifies the *F*-value of the group analysis. The degree of freedom for the *F* statistic for the right ventral anterior cingulate cortex (ACC) is 8.58. (B) Pearson correlation analysis between the individual mean fALFF value in the right ventral ACC (peak coordinate: 6, 30, −6; cluster size: 16) and HAMA scores in the pooled depression group. The dark-purple dots represent patients with remitted depression, and the light-purple dots represent patients with anxious depression. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

elucidate the pathophysiologic mechanisms underlying anxious depression.

The neural mechanisms of anxious depression are understudied in the field of neuropsychiatry. The limited studies that have been reported in the literature are not consistent in their definitions of anxious depression. Some of them used a dimensional definition (depression with high levels of anxiety symptoms), and some used a syndrome definition (diagnosis of major depression plus an anxiety disorder) [2,55]. Here, we studied anxious depression with the dimensional definition. The current study found that patients with anxious depression had altered intrinsic neural oscillations within corticolimbic circuits, including the insula and ventral ACC. The alterations in the right ACC and right dorsal anterior insula may play a critical role in the symptomatology of depression and anxiety, respectively. This hypothesis warrants future studies to investigate the possibility that these regions may be important biomarkers for the treatment of anxious depression.

Financial support

Conflict of interest

There are no conflicts of interest, financial or otherwise, related directly or indirectly to this work.
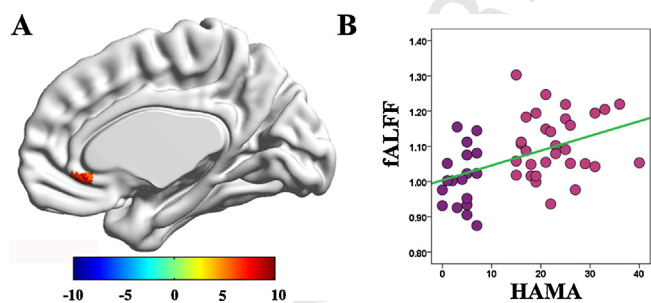
Ethical standards

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

Acknowledgements

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