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ORIGINAL RESEARCH

Amygdala connectivity mediates the association between anxiety and depression in patients with major depressive disorder

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Abstract

Anxiety is the most frequently co-occurring symptom with depression and subsequently contributes to increased severity and treatment resistance in patients with major depressive disorder (MDD). However, little is known about how these two behaviors are linked or interact at the neural network level. Seventy-five unmedicated MDD patients and 42 cognitively normal (CN) subjects underwent resting-state functional magnetic resonance imaging (R-fMRI) and neuropsychological testing. Multivariate linear regression analysis was performed to investigate the neural substrates of anxiety and depression, as well as their interactive effects on the amygdala functional connectivity (AFC) network in MDD patients. In addition, mediation analysis was employed to explore whether intrinsic amygdala connectivity mediates the association between anxiety and depression in patients with MDD. We found that MDD patients suffered symptoms of severe anxiety and a widely reduced functional connectivity in the AFC network, especially in the frontoparietal system and medial temporal lobe. Furthermore, common and distinct neural circuits involving anxiety and depression were separately identified. Interactive analysis revealed that MDD patients with lower HAMA scores showed milder depressive symptoms and greater AFC strength, while those with higher HAMA scores showed more severe depressive symptoms and lower AFC strength. More importantly, mediation analysis suggested that amygdala connectivity strength mediated the relationship between anxiety and depression in MDD patients. These findings extend our understanding of the brain circuitry implicated in MDD patients with comorbid anxiety, and provide new insight into therapeutic targeting of the neural circuits involved in this comorbidity.

Keywords Anxiety · Comorbidity · Intrinsic functional connectivity · Neural circuits · Amygdala · Major depressive disorder

Introduction

Major depressive disorder (MDD) is a highly prevalent psychological condition associated with substantial disability, morbidity, and mortality. Comorbid anxiety symptoms or anxiety disorders are common in patients with MDD, and it has been reported that these disorders may share an underlying pathophysiology. Combining dimensional and syndromal

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criteria, MDD with high levels of anxiety or with comorbid anxiety disorders has been defined as anxious depression. Recent studies have reported that patients with anxious depression exhibit severe depressive symptoms, greater functional impairment, increased incidence of suicidal ideation and suicide attempts, and poorer quality of life than those with non-anxious depression (Ormel et al. 1994; Roy-Byrne et al. 2000; McLaughlin et al. 2006; Fichter et al. 2010). Studies focused on the treatment of individuals with MDD have demonstrated that comorbid anxiety is associated with substantial resistance to treatment with antidepressants, an increased likelihood of adverse effects during treatment, lower rates of remission, and higher rates of recurrence (Jakubovski and Bloch 2014; Saveanu et al. 2015; Wiethoff et al. 2010; Goldberg and Fawcett 2012; Wu et al. 2013). These findings imply that MDD with comorbid anxiety-related characteristics is a more severe form of the disorder than either condition alone. Nevertheless, few studies have investigated the neural circuits involved in the comorbidity in MDD patients with anxiety. Clarifying the origins of this comorbidity and the underlying brain structures by which anxiety worsens depression may open new realms for understanding MDD pathophysiology, as well as providing a novel target for the effective treatment of patients with MDD.

The amygdala, as an important area for threat processing and orchestration of a complex set of emotional and physiologic responses, is centrally implicated in MDD (LeDoux 2000; Drevets 2003). Specifically, amygdala networks are involved in critical functions including the regulation of emotion, modulation of sensory information, and visceral response to negative stimuli (Price 2003). It is well documented that the disruption of amygdala connectivity is present in and contributes significantly to the severity of depression in patients with MDD (Xie et al. 2012b; Lui et al. 2011; Dannlowski et al. 2009). A recent functional magnetic resonance imaging (fMRI) study examined individuals with MDD, generalized anxiety disorder (GAD), and comorbid MDD-GAD, who completed an affective conflict resolution task; the authors found common anomalies in the amygdala under conditions of affective conflict in all subjects (Etkin and Schatzberg 2011). Another study showed that, relative to healthy participants, both MDD and anxiety patients exhibited amygdala hyperactivation in response to stimuli of negative facial expressions (Beesdo et al. 2009). Research on the neural circuitry of anxiety disorders has identified the importance of amygdala networks under emotional stimuli in animal models as well as in healthy humans (Shin and Liberzon 2010). Generally, prior studies have reported relatively heightened amygdala activation in response to negative stimuli in various categories of anxiety disorder (Blair et al. 2008; Hermann et al. 2007; Pillay et al. 2007; van den Heuvel et al. 2005; Wendt et al. 2008). Based on the crucial role of the amygdala in emotion systems and its implication in both MDD and anxiety disorder, the present study focused on examining the functional significance of the amygdala networks in MDD patients with different levels of anxiety.

Resting-state functional magnetic resonance imaging (R-fMRI) provides a powerful tool for probing the architectures of specific neural networks and their significance in healthy individuals and those with neuropsychiatric disorders (Poldrack and Farah 2015). Spontaneous fluctuation of the blood oxygen level-dependent (BOLD) signal is employed as an index to measure the mutual correlation between brain regions as resting-state functional connectivity (RSFC). Positive and negative correlations are used to denote neural synchrony in regions promoting similar and opposite goals, respectively. Recent neuroimaging studies have suggested that disruption of amygdala-frontal circuits is involved in the pathophysiology of MDD and anxiety disorder (Cullen et al. 2014; Carballedo et al. 2011; Gee et al. 2013; Holzel et al. 2013). Lower gray matter volumes of the rostral anterior cingulate gyrus (ACC) extending into the dorsal ACC (dACC) were also demonstrated in MDD patients with anxiety in a cross-sectional study (van Tol et al. 2010). Task-dependent fMRI studies revealed that, relative to healthy controls, both patients with MDD and those with anxiety exhibited hyperactivation of both the amygdala and ventral ACC in response to negative stimuli (Beesdo et al. 2009; Etkin and Schatzberg 2011), while specific compensatory activity of the dorsolateral prefrontal cortex (dlPFC) was seen in MDD (Etkin and Schatzberg 2011). In addition, heightened occipital cortex and anterior insula activity, as well as decreased default-mode network (DMN) response to criticism, were found only in patients with non-comorbid anxiety, and sustained medial frontal cortex activation was demonstrated in participants with non-comorbid MDD, while patients with comorbid MDD-GAD exhibited both of these patterns, as well as uniquely reduced dACC in response to praise (Waugh 2012; Hamilton et al. 2015). A recent R-fMRI study reported that RSFC of the limbic network (including the bilateral precuneus, intracalcarine cortex, lingual gyrus, and posterior cingulate, as well as the right precentral and middle frontal gyrus) was specifically increased in patients with comorbid MDD and anxiety, compared to healthy controls (Pannekoek et al. 2015). Taken together, these studies provide evidence that anxiety and depression both exhibit abnormalities mainly in limbic regions, such as the amygdala, as well as the prefrontal regions. In other words, limbic network abnormalities may be specifically associated with comorbid MDD and anxiety. These findings initially reveal the common and separate neural pathways involved in anxiety disorder or MDD, and provide direct evidence for the functional specialization in brain regions with regard to the effects of anxiety and MDD on behavioral modulation. The majority of this research, however, did not address the issue of this comorbidity, and the neural substrates potentially responsible for linking depressive

symptoms and anxiety severity in MDD patients are not yet fully understood.

The primary goal of the present study was to investigate differences in bilateral amygdala functional connectivity (AFC) networks between MDD patients and cognitively normal (CN) subjects at rest. We also examined the main and interactive effects of depression and anxiety on the bilateral AFC networks, as well as the behavioral significance of bilateral AFC networks, including depression severity and anxiety level, to reveal the brain-behavior relationship. Finally, we employed mediation analysis to determine the potential effects of intrinsic AFC strength in the modulation of anxiety and its relationship to the expression of depression in patients with MDD.

Materials and methods

All protocols were approved by the Medical Ethics Committee for Clinical Research of ZhongDa Hospital Affiliated with Southeast University. Written informed consent was obtained from all subjects prior to the study.

Participants

A total of 123 participants, including 80 patients with MDD and 43 CN subjects, were initially recruited into this study and underwent neuropsychological testing and MRI. MDD patients were recruited through the inpatient and outpatient department of psychiatry in ZhongDa Hospital affiliated with Southeast University, while CN subjects were enrolled through community posting and media advertising. All subjects were Chinese Hans and were right-handed. After excluding participants with excessive motion and/or incomplete echo-planar imaging (EPI) scans, as well as white matter hyperintensities, 75 unmedicated MDD patients and 42 CN subjects were included in our final analyses (see Table 1).

Inclusion and exclusion criteria

Criteria for inclusion in the MDD group included the following: individuals (1) met the diagnostic criteria for MDD using a structured clinical interview by two senior trained psychiatrists (Y. Yin and C. Xie) according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV); (2) received a score of > 17 on the Hamilton Depression Rating Scale (HAMD-17); (3) were medication-naïve; (4) were between 18 and 59 years of age, with age of onset < 55 years; and (5) had no contraindication to MRI scanning. Exclusion criteria for the MDD group were (1) other major psychiatric disorders or neurodegenerative illness history; (2) substance abuse (caffeine, nicotine, or alcohol), head trauma, or loss of consciousness; or (3) cardiac or pulmonary disease that could influence the BOLD response. CN subjects were required to have a Mini-Mental State Examination (MMSE) score ≥ 24 and HAMD-17 score of ≤ 7. Exclusion criteria for the CN group were a history of neuropsychiatric disease, head injury, drug abuse or insobriety, or ferrous or electronic implants.

Behavior measurements

All subjects underwent a comprehensive clinical assessment including demographic characteristics and medical history, as well as neurological and mental status examination. The HAMD was conducted for evaluation of depression, and the Hamilton Anxiety Rating Scale (HAMA) was used to assess anxiety severity.

Neuroimaging data acquisition

Data were acquired at the affiliated ZhongDa Hospital of Southeast University using a Siemens Verio 3.0-T scanner (Siemens, Erlangen, Germany) with a 12-channel head coil. The subjects were instructed to relax, close their eyes, and let their minds wander freely during the scan. Earplugs were used to reduce noise, and a pair of stabilizers to immobilize subjects' heads. Resting-state functional images, including 240 volumes, were obtained using gradient-recalled echo-planar imaging (GRE-EPI) sequences, as follows: repetition time (TR) = 2000 ms; echo time (TE) = 25 ms; flip angle (FA) = 90°; acquisition matrix = 64 × 64; field of view (FOV) = 240 × 240 mm; thickness = 4.0 mm; gap = 0 mm; number of slices = 36. The slices were acquired in an interleaved order (1, 3, 5 …, 35, 2, 4, 6 …, 36). High-resolution T1-weighted anatomical images covering the whole brain were acquired using a 3D magnetization-prepared rapid gradient-echo sequence: TR = 1900 ms; TE = 2.48 ms; FA = 9°; acquisition matrix = 256 × 256; FOV = 250 × 250 mm; thickness = 1.0 mm; gap = 0 mm, number of slices = 176. Additionally, routine axial T2-weighted images were obtained to exclude subjects with major white matter (WM) changes, cerebral infarction, or other lesions.

Image preprocessing

Data preprocessing of subjects was conducted using the SPM8 toolkit (http://www.fil.ion.ucl.ac.uk/spm), and data were analyzed using the Analysis of Functional

Table 1 Demographic information and behavior data

	CN ($n = 42$)	MDD ($n = 75$)	T or χ^2	p value
Age (years)	41.31 ± 11.65	40.41 ± 11.59	0.40	0.96
Gender (M/F)	23/19	33/42	1.25	0.33[a]
Education (years)	11.67 ± 4.27	9.77 ± 3.59	2.55	0.01
Age of onset (years)	NA	33.67 ± 13.04	NA	NA
Course of disease (years)	NA	7.44 ± 8.28	NA	NA
Number of episodes	NA	2.81 ± 1.92	NA	NA
Gray matter volume (ml)	647.04 ± 63.19	620.28 ± 64.24	2.17	0.03
HAMD	1.10 ± 1.56	21.29 ± 5.12	24.88	0.00
HAMA	1.43 ± 2.04	17.01 ± 6.54	15.03	0.00
High-HAMA (%)	0.00%	45.33%	NA	NA

[a] p value was obtained by chi-square test; other p values were obtained by two-way t test. *SD* standard deviation, *M* male, *F* female, *CN* cognitively normal, *MDD* major depressive disorder, *HAMD* Hamilton Depression Rating Scale (17-item), *HAMA* Hamilton Anxiety Rating Scale, *High-HAMA* HAMA score > 16

NeuroImages (AFNI) (http://afni.nimh.nih.gov/afni) and MATLAB version 7.10 (The MathWorks, Inc., Natick, MA, USA) software programs. The first ten volumes of the scanning session were discarded to allow for T1 equilibration effects. The remaining 230 volumes were corrected for slice timings, realigned [participants with head motion > 2.5 mm maximum displacement in any direction (x, y, z) or 2.5° of angular motion were excluded). No significant differences in head motion were found between the two groups ($p > 0.05$). The resulting images were spatially normalized to the standard Montreal Neurological Institute (MNI) EPI template using the default settings, resampled to $3 \times 3 \times 3$ mm^3 voxels, and smoothed with a Gaussian kernel of $6 \times 6 \times 6$ mm. Finally, a band-pass filter was applied to keep only low-frequency fluctuations between 0.01 and 0.08 Hz.

Amygdala functional connectivity analysis

We used an anatomically based region-of-interest (ROI) method to extract the bilateral amygdala as seed regions from the Harvard-Oxford subcortical atlas (Desikan et al. 2006). The time course of the bilateral amygdala regions was extracted from the functional EPI image for each subject. The averaged time course of each amygdala region, as the seed time course, was then correlated with the time courses of all brain voxels using Pearson cross-correlation. Next, Pearson correlation coefficients (r) were applied to Fisher z transformation analysis, which yielded variants of approximately normal distribution [$m = 0.5\ln(1 + r)/(1 - r)$] (Zar 1996). Finally, the data were spatially normalized to the MNI space, resampled to 3-mm isotropic voxels, and smoothed with a Gaussian kernel (6 mm full width at half maximum).

Structural image analysis

To avoid the bias of functional connectivity strength due to anatomical variation, gray matter (GM) volume was considered as an important covariate in the functional connectivity analysis (Xie et al. 2011; Xie et al. 2015). Optimized voxel-based morphometry (VBM) analysis was performed, using the VBM8 toolbox in SPM8 to calculate GM volume in all subjects. The T1-weighted images were segmented to GM, WM, and CSF, after which the segmented GM was normalized and smoothed as the functional image. The final images were regressed out as covariates of no interest to control the effect of GM volume on intrinsic functional connectivity strength.

Statistical analysis

Demographic and behavior data

Independent t tests and chi-square tests (only for gender) were used to compare the demographic data and behavioral performance between the two groups. Statistical significance was set at $p < 0.05$. All statistical analysis utilized SPSS version 20.0 software (IBM Corp., Armonk, NY, USA).

Resting-state functional connectivity analysis

First, one-sample t test was used to obtain individual AFC pattern in each group respectively, after corrected with the new 3dClustSim program. Two-sample t tests were then used separately to obtain the group difference for each AFC network after removing the effects of covariates including gender, age, education, and GM volumes (3dttest^{++}, AFNI, corrected with 3dClustSim, $p < 0.001$). Next, to investigate the behavioral significance of bilateral AFC networks in the

MDD patients, multivariate linear regression analysis was employed, as follows:

$$m_i = \beta_0 + \beta_1 *Anx + \beta_2 *Dep + \beta_3 *Anx*Dep + \beta_4 *Edu + \beta_5 *Gen + \beta_6 *Age + \beta_7 *GMV + \epsilon$$

where m_i is the connectivity strength of the voxel in the left and right AFC networks for individual subjects, *Anx* is the HAMA score, and *Dep* is the HAMD score in each subject. *GMV* is the total GM volume for each subject. β_0 is the intercept of straight line fitting in the model; β_1, β_2, and β_3 are the effects of anxiety, depression, and their interaction on the functional connectivity strength of the voxel in the left and right amygdala networks, respectively; β_4, β_5, β_6, and β_7 are the effects of education, gender, age, and GM volumes as no-interest covariates in the above model; ϵ is the model error. Cluster-level thresholds corrected for multiple comparisons were derived using Monte Carlo simulation of the random noise distribution in the data after correction using the latest version of the 3dClustSim program in AFNI version 16.3.00 [gray matter mask correction (67,541 voxels), voxel level $p < 0.05$, cluster level $\alpha < 0.001$, $\kappa > 146$, cluster size > 3942 mm^3; https://afni.nimh.nih.gov/pub/dist/doc/program_help/3dClustSim.html].

Mediation analysis

Given the significant influence of anxiety symptoms and AFC strength on the severity of depression observed in MDD patients, we performed mediation analysis to further determine whether AFC strength could mediate the association between anxiety and depression severity in patients with MDD. This approach is based on a standard three-variable mediation model and is in line with the currently most widely accepted mediation analysis technique (Ma et al. 2014; Bolin 2014). A detailed description of the method is provided in the supplementary material.

Results

Demographic information and behavior data

Demographic data and behavior performance for the two groups are shown in Table 1. There were no significant differences between CN subjects and MDD patients with respect to age or gender. As expected, the groups differed significantly with respect to HAMD and HAMA scores. Specifically, MDD patients showed lower levels of education and GM volumes than CN subjects, while presenting higher severity of depression and anxiety. The MDD subjects were further divided into two groups. Thirty-four subjects with HAMA scores > 16 were defined as the high-HAMA group, which constituted 45.33% of the total MDD patients.

Resting-state AFC network patterns in CN and MDD subjects

Resting-state AFC maps in the CN subjects and MDD patients ($p < 0.01$, with cluster size > 450 mm^3) are briefly illustrated in Fig. S1. Generally, each network was composed of the positive network and anticorrelated (negative) network, and the strongest connectivity of each network was adjacent to its seed amygdala. Non-adjacent regions also showed significant connectivity associated with each seed amygdala (detailed information described in supplementary material).

Group-level differences in amygdala functional connectivity network

Compared with CN subjects, patients with MDD showed widespread decreased functional connectivity in the bilateral AFC networks, including the bilateral insula lobe, hippocampus, temporal pole (TP), supramarginal gyrus (SMG), dorsomedial prefrontal cortex (dmPFC), left fusiform area (FFA) and inferior temporal gyrus (ITG), dlPFC, inferior parietal lobule (IPL) and parahippocampal gyrus (PHG), and even reversed from negative to positive connectivity in the bilateral medial prefrontal cortex (mPFC), while no regions of increased AFC were found, as shown in Fig. 1 and Table S1.

Behavioral significance of bilateral AFC networks

Multivariate linear regression analysis revealed that the main effects of depression on the bilateral AFC networks were located primarily in regions including the bilateral middle temporal gyrus (MTG) and lingual gyrus (LG), the left superior temporal gyrus (STG), FFA, orbitofrontal cortex (OFC) and caudate, and the right insula lobe, inferior frontal gyrus (IFG), dmPFC, and TP (Fig. 2a). The main effects of anxiety on the bilateral AFC networks were observed in clusters that consisted of the bilateral dmPFC, FFA and PCC, left MTG and middle cingulate cortex (MCC), and the right TP (Fig. 2b).

Interactive effects of anxiety and depression on AFC networks

The interactive effects of anxiety and depression on the bilateral AFC networks were also found in the bilateral mOFC, left MTG and FFA, and right TP, dmPFC, and LG (Fig. 3a). In order to depict the specific interactive pattern of anxiety and depression with regard to the AFC networks, we divided

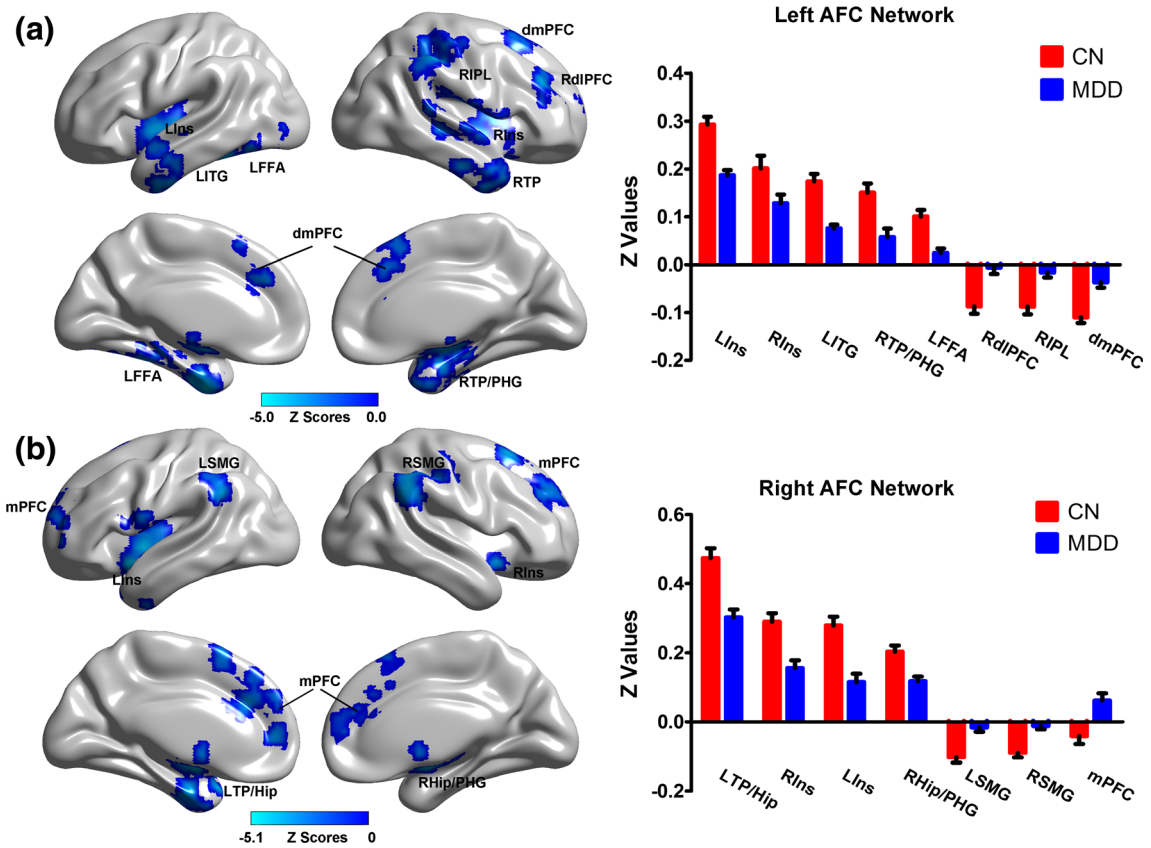

Fig. 1 Differential bilateral amygdala functional connectivity (AFC) networks in MDD patients compared with CN subjects. The results illustrate the differential functional connectivity of the left AFC (**a**) and right AFC (**b**) networks in MDD patients compared with CN subjects. Blue indicates decreased connectivity. *Z* scores are indicated by the color bar. In addition, a numerical representation of significant differences in functional connectivity within the bilateral AFC networks between the two groups is provided by the histograms in (**a**) and (**b**). Abbreviations: AFC, amygdala functional connectivity; CN, cognitively normal; MDD, major depressive disorder; LIns, left insula; RIns, right insula; LITG, left inferior temporal gyrus; RTP, right temporal pole; RPHG, right parahippocampal gyrus; LFFA, left fusiform area; RdlPFC, right dorsolateral prefrontal cortex; RIPL, right inferior parietal lobe; dmPFC, dorsomedial prefrontal cortex; LHip, left hippocampus; LTP, left temporal pole; RHip, right hippocampus; LSMG, left supramarginal gyrus; RSMG, right supramarginal gyrus; mPFC, medial prefrontal cortex

MDD patients into a high-anxiety (34 patients, HAMA scores > 16) and a mild-anxiety group (35 patients, HAMA scores ≤ 16). Further analysis revealed that MDD patients with lower HAMA scores showed milder depressive symptoms and greater AFC strength (Fig. 3b, left and middle columns) or more negative AFC strength (close to CN subjects, Fig. 3b, right column), while MDD patients with higher HAMA scores showed more severe depressive symptoms, with weaker AFC strength (Fig. 3b, left and middle columns), or far from normal values (Fig. 3b, right column). Importantly, the associated AFC regions, including the left TP and FFA and the right dmPFC, also overlapped with the altered regions in MDD patients.

Mediation analysis

Exploratory mediation analyses revealed that AFC in brain regions, which showed the interactive effects of anxiety and depression on the bilateral AFC networks, could modulate the association between anxiety and depression, except for the left FFA region (Fig. 4). Specifically, negative correlations were found between anxiety and the AFC networks in four regions (path *a*, Fig. 4, left and middle columns, $p < 0.001$), while positive correlations were found in the other two regions (path *a*, Fig. 4, right column, $p < 0.001$). For path *b*, we observed parallel outcomes. These findings indicate that intrinsic AFC strength leads to a significant positive (path *a* by path *b*) mediation effect between HAMA scores and the severity of depression in all interactive regions except the left FFA region. Together, these post hoc analyses directly support the notion that the relationship between depression and anxiety may be mediated through the intrinsic AFC network.

Discussion

In the current study, three major findings were generated. First, disruption of the intrinsic AFC network was found in

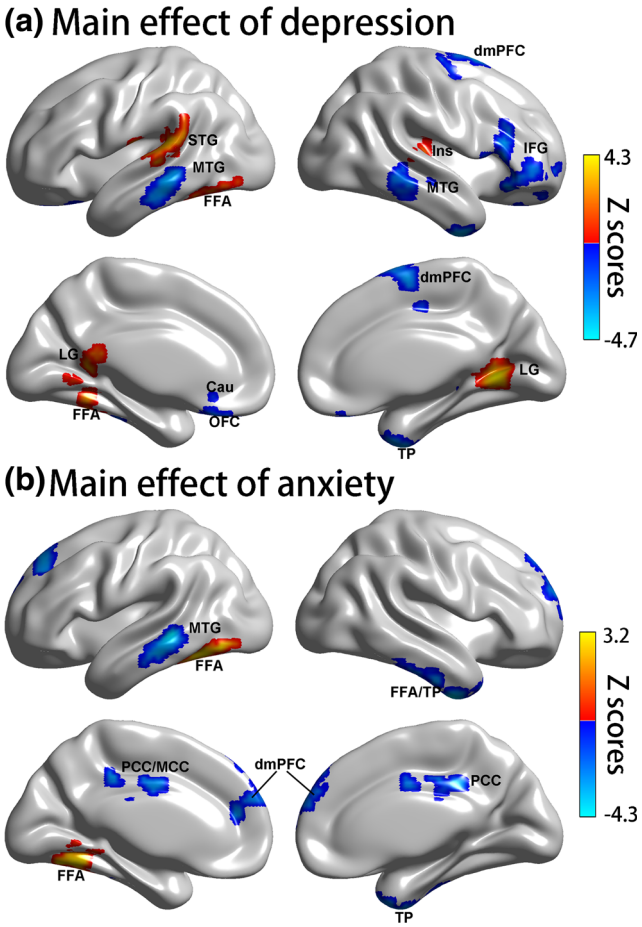

Fig. 2 Neural basis of depression and anxiety with regard to the amygdala functional connectivity (AFC) networks in MDD patients. The results show brain regions with significant effects of depression (**a**) and anxiety (**b**) on AFC networks. Red indicates positive correlation, and blue indicates negative correlation. Color bar is presented with Z scores. Abbreviation: STG, superior temporal gyrus; MTG, middle temporal gyrus; FFA, fusiform area; dmPFC, dorsomedial prefrontal cortex; IFG, inferior frontal gyrus; Ins, insula; LG, lingual gyrus; OFC, orbitofrontal cortex; Cau, caudate; TP, temporal pole; PCC, posterior cingulate cortex; MCC, middle cingulate cortex

MDD patients compared to CN subjects, located primarily in the frontal-parietal cortex, temporal gyrus, and subcortical regions. Second, we identified that distinct regions of the cortico-amygdala pathway were separately involved in high and mild anxiety among patients with MDD. Third, mediation analysis further demonstrated that intrinsic AFC strength could indirectly mediate the relationship between anxiety and depression. However, this does not imply a causal relationship between amygdala connectivity strength and severity of anxiety and depression in MDD. In summary, these findings provide direct empirical evidence for the neural basis of comorbid anxiety and depression in patients with MDD, and extend our understanding of how intrinsic brain function influences the specificity of the behavioral phenotypes.

Altered intrinsic AFC network connectivity in MDD patients

It is well established that the amygdala and the prefrontal-parietal cortex (including the dlPFC, ventrolateral PFC, dorsal/middle cingulate, PCC, OFC, SMG) are tightly connected in structure and functionally involved in decision-making, emotion, and memory processing (Andersen and Cui 2009; Etkin et al. 2009; Spielberg et al. 2008). Previous task-dependent fMRI studies have revealed reduced BOLD activation in the right frontoparietal network in MDD patients when performing the Stroop task under incongruent conditions (Wagner et al. 2015). Meta-analyses of fMRI studies have demonstrated that depressed patients display abnormal activation of the ACC, prefrontal cortex (PFC), amygdala, thalamus/basal ganglia, and hippocampus (Jaworska et al. 2015; Kong et al. 2013). A recent study identified altered amygdala connectivity to cognitive control or affective networks in MDD patients, suggesting multiple sources of dysregulation in the complex brain systems implicated in dysfunctional emotion-cognition integration in these patients (Wang et al. 2016). Therefore, the decoupling of amygdala connectivity to the frontal-parietal network may indicate that MDD patients fail to compete for neuronal resource allocation with cognitive control processes, leading to deficient cognitive performance (Wagner et al. 2015). In addition, it is well known that the insula, as a key node subserving cognition-emotion integration, has been proven to participate in the perception of pain (Lamm et al. 2011), empathy and social emotions (Lamm and Singer 2010), subjective awareness (Craig 2009), polymodal sensory integration (Critchley et al. 2004), and even episodic memory (Xie et al. 2012a). Decreased regional homogeneity (ReHo) in the right insula, which is a measure of regional brain activity, was also reported in patients with MDD and those at high risk for MDD (Liu et al. 2010), and correlated positively with anxiety severity (Yao et al. 2009). A task-dependent fMRI study detected hypoactivation of the right insula in response to negative affective pictures in depressed individuals (Lee et al. 2007), and this hypoactivation was closely associated with anhedonia during reward processing in MDD patients (Miller et al. 2015). Researchers have recently identified the insula and its functional connectivity to be associated with abnormal interoceptive activity, higher levels of recent rumination, and severity of depression in MDD patients (Avery et al. 2014; Kaiser et al. 2015; Henje Blom et al. 2015). In addition, two resting-state PET studies found that the distribution of serotonin receptor 2 (5-HT2) was significantly reduced in the anterior insula cortex in patients with depression, and the binding potential (BP) of the serotonin transporter (5-HTT) has been found to be increased in the insula (Yao et al. 2009; Cannon et al. 2007). Taken together, the findings of reduced intrinsic AFC predominantly with regions implicated in emotional

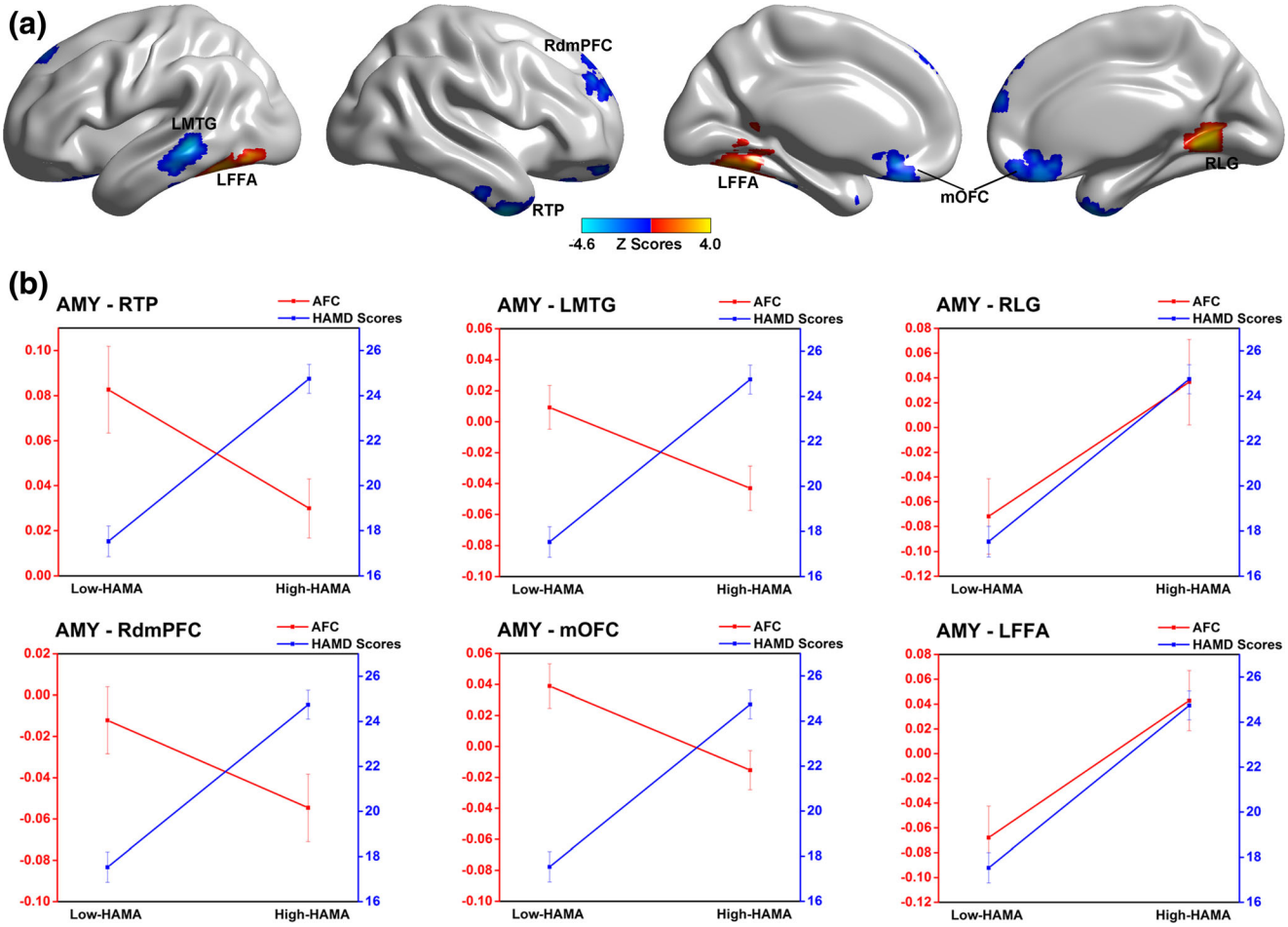

Fig. 3 **a** Interactive effects of anxiety and depression on the bilateral amygdala functional connectivity (AFC) networks in MDD patients. Red indicates positive correlation, and blue indicates negative correlation. *Z* scores are indicated by the color bar. **b** Numerical representation of the relationships among anxiety, depression, and amygdala connectivity. *Left and middle rows:* MDD patients with lower HAMA scores showed milder depressive symptoms and greater AFC strength, while MDD patients with higher HAMA scores showed more severe depressive symptoms, with decreased AFC strength. *Right row:* MDD patients with lower HAMA scores showed milder depressive symptoms and more negative AFC strength (close to CN subjects), while MDD patients with higher HAMA scores showed more severe depressive symptoms, with weaken AFC strength (far from CN subjects). Red = *z* values of amygdala functional connectivity; blue = HAMD scores. Abbreviations: AMY, amygdala; AFC, amygdala functional connectivity; HAMD, Hamilton depression rating scale; HAMA, Hamilton Anxiety Scale; RTP, right temporal pole; RdmPFC, right dorsomedial prefrontal cortex; LMTG, left middle temporal gyrus; mOFC, medial orbitofrontal cortex; RLG, right lingual gyrus; LFFA, left fusiform area

and cognitive processing may represent evidence of impaired interceptive activity signaling for high-order cortical modulation of limbic regions leading to abnormal affective and cognitive regulation in patients with MDD.

Another new finding demonstrated in the present study was that amygdala–mPFC connectivity was decreased in MDD patients. As a key node of the DMN, mPFC is known to be involved in many seemingly different functions, including autobiographical information, self-reference, emotion, cognitive function, and memory (Andrews-Hanna et al. 2014; Weiss-Croft and Baldeweg 2015). Our current finding indicates the heterogeneous properties of the DMN, and thus suggests that disruption of the sub-regional DMN network may be implicated in the different clinical symptoms present in MDD patients.

Relationship between the intrinsic AFC network and depressive symptoms

Numerous fMRI-based studies have identified disruption of intrinsic connectivity associated with depressive symptoms (Xie et al. 2012b; Zeng et al. 2012). For example, task-dependent fMRI studies in depressed patients demonstrated a reciprocal relationship between decreased activation in the dorsal and ventral parts of the PFC and dACC regions, and increased activation in the limbic/paralimbic areas, which include the amygdala, hippocampus, thalamus, and basal ganglia. R-fMRI studies have also shown that disruption of the amygdala network is responsible for dysfunctional affective and cognitive processing in MDD patients (Kong et al. 2013; Ramasubbu et al. 2014; Yue et al. 2013). In addition, a

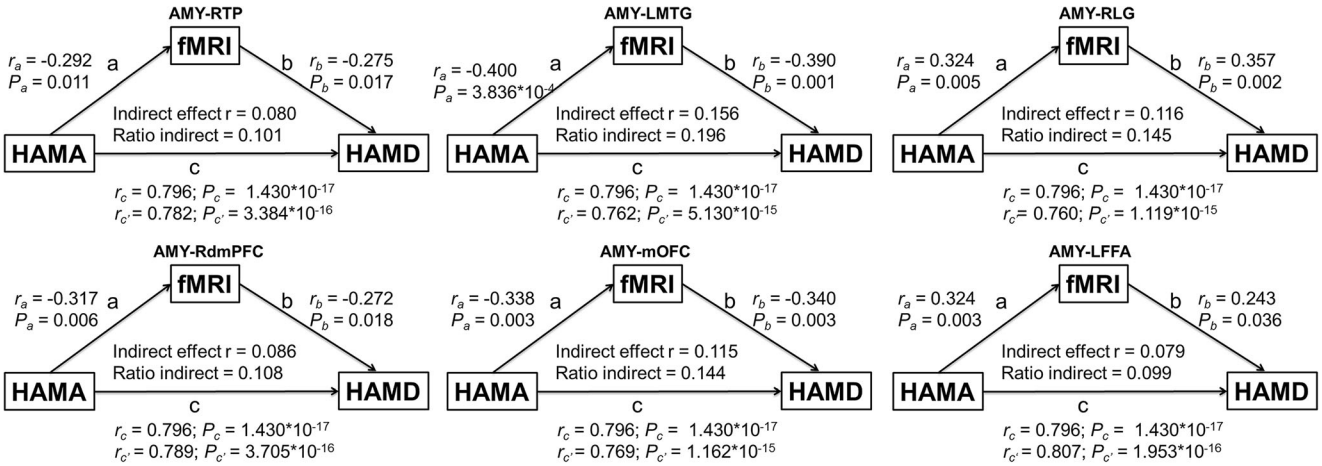

Fig. 4 Mediation effects of amygdala functional connectivity on the association between anxiety and depression in MDD patients. The results of mediation analysis reveal that the AFC was able to modulate the association between anxiety and depression, except in the LFFA region. Abbreviations: AMY, amygdala; AFC, amygdala functional connectivity; HAMD, Hamilton depression rating scale; HAMA, Hamilton Anxiety Scale; fMRI, functional magnetic resonance imaging; RTP, right temporal pole; RdmPFC, right dorsomedial prefrontal cortex; LMTG, left middle temporal gyrus; mOFC, medial orbitofrontal cortex; RLG, right lingual gyrus; LFFA, left fusiform area

multivariate pattern analysis using whole-brain functional connectivity revealed that the amygdala, ACC, parahippocampal gyrus, and hippocampus exhibited high discriminative power in differentiating MDD patients from CN subjects, and suggests that functional coupling of these regions may play an important role in the pathological mechanism of MDD (Zeng et al. 2012). These studies would thus indicate that the AFC network is crucial for emotion processing, especially in the presence of depressive symptoms.

Relationship between the intrinsic AFC network and anxiety symptoms

Multivariate linear regression analysis was also performed with HAMA scores and identified the neural basis of anxiety. Two prior task-dependent fMRI studies conducted in adolescents with GAD found evidence of hyperactivity in the amygdala (Monk et al. 2008; McClure et al. 2007), while R-fMRI research identified abnormal amygdala connectivity and engagement of a compensatory frontoparietal executive control network in adult GAD patients (Etkin et al. 2009). Furthermore, accumulating evidence has demonstrated that diminished functional connectivity in the amygdala-ventral PFC pathway is associated with less habituation over repeated exposures, and the tight relationship between the amygdala and mPFC is thought to be important for regulation of emotion through cognitive control (Johnstone et al. 2007; Urry et al. 2006; Ochsner and Gross 2005; Wager et al. 2008; Kim et al. 2011). It has also been reported that the connectivity strength of the amygdala-PFC pathway was inversely correlated with trait anxiety levels in healthy subjects (Kim and Whalen 2009; Hare et al. 2008), while patients with social anxiety disorder exhibited decreased functional coupling of the amygdala with the PCC/Pcu, which also negatively correlated with the severity of the state of anxiety, indicating a modulatory influence of the amygdala-PCC pathway on threat perception processing (Hahn et al. 2011). Taken together, these findings revealed a functional specialization within the amygdala network for anxiety disorder, and also suggested that the distinctive change in the AFC network observed in this study is related to anxiety symptoms.

Neural basis for the interactive effects of depression and anxiety on the AFC network

In the current work, the dual functions of the amygdala network were found to be involved in both depressive symptoms and anxiety disorder, which strongly supports a recent conceptual hypothesis proposing that complex cognitive and emotional behaviors have their neural bases in the dynamic coalition of networks with distributed brain regions (Pessoa 2008). Importantly, the interactive neural basis of symptoms of depression and anxiety were mediated by altering the functional connectivity between amygdala and specific brain regions (including bilateral mOFC, right TP, dmPFC and LG, and left MTG and FFA), which are closely associated with cognitive control and emotion processing (Janak and Tye 2015; Hirayama 2015). Anxiety- and depression-relevant processes, such as reward and motivation, affect regulation, threat processing, and memory, consistently implicating regions such as the amygdala, ventral striatum, hippocampus, and subgenual ACC. These regions are most important for comprehending both categorical and dimensional aspects of anxiety and depression. A recent study using structural MRI found areas of atrophy involving the right OFC, FFA, and rostral ACC, the left TP and occipital cortices, and

the bilateral precentral gyrus, parietal lobe, and precuneus in MDD-comorbid GAD patients (Canu et al. 2015). The engagement of amygdala connectivity with these structures enables a detailed appraisal and conscious awareness of the emotional stimulus, and in turn these regions provide feedback for context-appropriate regulation, which may result in inhibition or enhancement of limbic processing. In MDD-GAD patients, these circuits cannot operate normally, leading to dysregulation of emotional stimuli and severe symptoms. Interestingly, Oathes and colleagues used R-fMRI to investigate the neurobiological signatures of anxiety and MDD and found that anxious arousal was also associated with lower amygdala connectivity to a specific target (mPFC), and MDD was associated with greater amygdala-to-subcortical connectivity. In addition, they demonstrated that in terms of functional connectivity, for the subgenual ACC (sgACC) area, patients with anxious arousal (GAD, GAD-MDD) showed changes opposite those in MDD patients. We also found negative amygdala-sgACC (within the mOFC cluster) connectivity in MDD patients with comorbid anxiety. Taken together, these findings suggest that neither a categorical nor a dimensional model of anxiety or depression alone can explain the neurobiology of this comorbidity. For further research, mapping the affective pathology onto neurobiological substrates requires combining categorical and dimensional specifications of anxiety and depressive disorders (Oathes et al. 2015). Task-dependent fMRI research has proven that patients with comorbid MDD and anxiety exhibit enhanced amygdala and OFC activation relative to healthy subjects when viewing facial expressions (neutral, fear, angry, and happy) (Beesdo et al. 2009). We showed decreased amygdala-OFC connectivity in comorbid MDD-anxiety patients. In particular, it is interesting to note the synergistic effect of the severity of anxiety and depression leading to AFC disruption, which exerts a substantial influence on the specificity of the behavioral phenotypes. Moreover, the engagement of cognitive control and emotional circuitry in MDD patients represents the complex nature of psychopathology arising from the interaction of anxiety and depression, all of which can occur at the neural network level. More importantly, it has been reported that gene polymorphisms involved in serotonin biosynthesis, transport, or signaling, as well as neurotrophins such as brain-derived neurotrophic factor, are associated with an elevated risk for depression and can result in differences in amygdala activation, structure, and connectivity (Scharinger et al. 2010; Munafo et al. 2008; Roiser et al. 2009). To further verify the relationship among depressive symptoms, anxiety symptoms, and AFC networks, mediation analysis was employed (Wager et al. 2008). We demonstrated that anxiety affected the expression of depression indirectly through intrinsic amygdala connectivity, which suggests that intrinsic AFC may act as a causal intermediate in the relationship between anxiety and depression.

Our study also has several limitations. First, this was a cross-sectional exploratory study, and the control subjects were not well matched with the patient group (education level in MDD was lower than that in CN group). To avoid the effects of age, gender, education level, and gray matter volume, we excluded these factors as covariates of no interest throughout the statistical analysis. Second, according to McDonald (1998), the amygdala can be subdivided into the superficial (SFA), centromedial (CMA), and basolateral (BLA), each with distinct functional connections to the other brain regions supporting different brain functions (McDonald 1998; Roy et al. 2009). Further studies should investigate the functional connectivity of the subdivisions of the amygdala. Third, a recent study revealed that amygdala activation measured by fMRI may be confounded by stimulus-related signal fluctuation in nearby veins draining distant brain regions (Boubela et al. 2015), thus raising concerns about many conclusions regarding the functioning of the amygdala that rely on fMRI evidence alone. Fourth, according to previous research, physiological noise can affect the resting-state signal and functional connectivity results to a certain degree (Birn 2012). Although we applied a band-pass filter (0.01–0.08 Hz) to roughly control the influence of physiological noise, the effects of physiological noise cannot be removed precisely. Finally, given the complex nature of co-occurring anxiety symptoms and depressive disorder, the clinical diagnostic process should consider that symptoms of anxiety in patients with MDD may differ from those in anxiety patients without MDD. Similarly, depressive symptoms in MDD patients with GAD may be different from those in patients without GAD. As suggested, future studies should assess anxiety disorder in the context of depression, and depression in the context of anxiety disorder.

Conclusions

In summary, we have demonstrated that the neural basis linking symptoms of depression and anxiety in MDD patients and intrinsic AFC strength could indirectly mediate the relationship between anxiety and depression. These findings extend our understanding of the brain circuitry implicated in MDD patients with comorbid anxiety, and provide new insight into potential therapeutic targeting of the neural circuits involved in this comorbidity.

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Compliance with ethical standards

Conflict of interest All authors of this paper declare that they have no potential conflict of interest, financial or otherwise, related directly or indirectly to this work.

Ethical approval All protocols were approved by the Medical Ethics Committee for Clinical Research of ZhongDa Hospital Affiliated with Southeast University. Written informed consent was obtained from all subjects prior to the study.

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