Differential Alterations of Resting-State Functional Connectivity in Generalized Anxiety Disorder and Panic Disorder

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Abstract: Generalized anxiety disorder (GAD) and panic disorder (PD) are most common anxiety disorders with high lifetime prevalence while the pathophysiology and disease-specific alterations still remain largely unclear. Few studies have taken a whole-brain perspective in the functional connectivity (FC) analysis of these two disorders in resting state. It limits the ability to identify regionally and psychopathologically specific network abnormalities with their subsequent use as diagnostic marker and novel treatment strategy. The whole brain FC using a novel FC metric was compared, that is, scaled correlation, which they demonstrated to be a reliable FC statistics, but have higher statistical power in two-sample t-test of whole brain FC analysis. About 21 GAD and 18 PD patients were compared with 22 matched control subjects during resting-state, respectively. It was found that GAD patients demonstrated increased FC between hippocampus/parahippocampus and fusiform gyrus among the most significantly changed FC, while PD was mainly associated with greater FC between somatosensory cortex and thalamus. Besides such regional specificity, it was observed that psychopathological specificity in that the disrupted FC pattern in PD and GAD correlated with their respective symptom severity. The findings suggested that the increased FC between hippocampus/parahippocampus and fusiform gyrus in GAD were mainly associated with a fear generalization related neural circuit, while the greater FC between somatosensory cortex and thalamus in PD were more likely linked to interoceptive processing. Due to the observed regional and psychopathological specificity, their findings bear important clinical implications for the potential treatment strategy. *Hum Brain Mapp 00:000–000, 2016. © 2016 Wiley Periodicals, Inc.*

Key words: anxiety disorders; functional magnetic resonance imaging (fMRI); fear; generalization; interoception; whole brain functional connectivity analysis

INTRODUCTION

Anxiety disorders are highly prevalent [Kessler et al., 1994, 2005a,b] and affect many aspects of daily life. Patients with anxiety disorders exhibit reduced quality of life, impaired social functioning, and higher morbidity and mortality [Rodriguez et al., 2005; Wittchen and Fehm, 2001]. Generalized anxiety disorder (GAD) and panic disorder (PD) are two common subtypes of anxiety disorders. GAD has the central defining feature of chronic, excessive anxiety and worrying, as described in the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5). Previous research has linked various fear-learning processes to GAD, including fear generalization [Cha et al., 2014a,b; Greenberg et al., 2013; Lissek et al., 2014; Mineka and Zinbarg, 2006]. Fear generalization is the transfer of conditioned fear to perceptually similar stimuli [Greenberg et al., 2013] and may contribute to an increase in the number of cues/events capable of triggering worry [Borkovec et al., 1991]. PD is characterized by recurrent unexpected panic attacks [American Psychiatric Association, 2013] and considerable studies have reported somatic abnormalities in PD, such as increased heart rate, higher skin conductance and increased respiration value [Fleet et al., 1996; Friedman and Thayer, 1998; Hoehn-Saric et al., 2004; Stein and Asmundson, 1994]. Studies suggested that these somatic symptoms in PD reflect abnormal interoceptive processing including visceroreception (from inner organs) as well as proprioception (from musculoskeletal organs) [Domschke et al., 2010; Ehlers and Breuer, 1992; Qiang et al., 2012; Van der Does et al., 1997; Willem Van der Does et al., 2000].

Imaging studies in GAD showed structural abnormalities in various regions including cingulate cortex, precuneal gyrus, precuneus, temporal, and frontal gyrus [De Bellis et al., 2002; Strawn et al., 2013; Zhang et al., 2013]. Task-related activity and connectivity changes were reported in parietal cortices (in an affective Stroop task) [Blair et al., 2012], Broca’s area and the occipitotemporal area (during a verbal fluency task) [Kalk et al., 2012], the putamen (during anticipation) [GUYER et al., 2012], the bed nucleus of the stria terminalis (BNST) (during uncertainty) [Yassa et al., 2012], the amygdala, the prefrontal cortex (PFC) and the insula, respectively, related to the specific paradigms used [Etkin et al., 2009, 2010; Etkin and Schatzberg, 2011; McClure et al., 2007; Roy et al., 2013; Strawn et al., 2012]. Resting state studies mainly concentrate in amygdala circuits involved in emotion processing [Etkin et al., 2009; Liu et al., 2015; Roy et al., 2013]. It remains unclear though whether these changes are specific for GAD in particular or are rather related to anxiety in general. Accordingly, comparison of resting state FC between different subgroups of anxiety disorders like GAD and PD is warranted.

Structure neuroimaging data in PD showed changes in amygdale [Hayano et al., 2009; Massana et al., 2003b] and other subcortical structures, such as parahippocampus gyrus, caudate nucleus, basal ganglion, insula [LAI 2011; Massana et al., 2003a; Uchida et al., 2008], and cortical areas like anterior cingulated cortex (ACC), frontal and...
Functional Connectivity in GAD and PD

Temporal areas [Asami et al., 2008; Fontaine et al., 1990; Han et al., 2008; Sobanski et al., 2010; Uchida et al., 2003]. Recent task-related functional magnetic resonance imaging (fMRI) studies suggested that increased activation in fear system structures like amygdala, insula, and hippocampus during agoraphobia-specific stimuli [Wittmann et al., 2011], and increased activation in the right inferior frontal area, the cingulate cortex during panic anticipation and imagery exposure [Bystritsky et al., 2001]. Investigations about resting-state in PD suggested abnormal FC within the salience network [Pannekoek et al., 2013] and default mode network (DMN) [Shin et al., 2013]. However, whether these resting state FC are regionally and psychopathologically specific to PD as distinguished from for instance GAD remains unclear at this point.

To address both regional and psychopathological specificity, we here investigated and respectively compared resting-state FC between GAD and healthy controls (HC), and between PD and HC. The resting-state functional connectival analysis is a sound approach in the neuropsychiatric disorders [Chen et al., 2015; Duncan et al., 2014; Luo et al., 2011; Northoff 2013]. Based on prior studies, we hypothesized that the FC among GAD and PD were associated with different neural circuits. Specifically, we expected to find altered FC in network involved in fear and generalization among GAD. For PD network we expected an abnormal perception of bodily signals like areas involved in somatosensory processing. In addition, there might be correlations between abnormal FC and disease severity. In order to avoid the confounding effects of medication, we only included drug-free (or drug-naive) patients in our study.

Unlike previous studies [Blair et al., 2012; Bystritsky et al., 2001; Etkin et al., 2009, 2010; Etkin and Schatzberg, 2011; Guyer et al., 2012; Kalk et al., 2012; McClure et al., 2007; Pannekoek et al., 2013; Roy et al., 2013; Straw et al., 2012; Wittmann et al., 2011] that mostly focused on ROIs defined as a priori, we relied on a whole-brain approach, that is, Brain-wide Association Study (BWAS) [Tao et al., 2013] to identify those networks that are specifically associated with GAD as distinguished from HC and with PD as distinguished from HC from the whole brain FC. BWAS is totally data-driven therefore avoids the shortcomings of ROI-based approaches. However, with whole brain analysis the traditional FC metric such as Pearson correlation may not provide enough statistical power required for multiple comparison, especially when the number of subjects enrolled is not large, like the current study. To obtain statistically significant results in datasets with relatively small sample size, we proposed to use a novel FC metric, that is, scaled correlation [Folias et al., 2013; Nikolic et al., 2012] in this article, which we demonstrate to have higher statistical power in two sample t-test of whole brain analysis than traditional Pearson correlation. This is because scaled-correlation can reduce the variance of FC distribution, with the mean of FC distribution unchanged compared with that from Pearson correlation. Therefore, the estimate of $P$ value in two sample $t$-test becomes more significant than that from Pearson correlation. Our analysis was finally complemented by correlating psychopathological scores of GAD and PD with the respective FC changes.

**METHOD**

**Subjects**

Sixty-one right-handed subjects were included in the study: 21 subjects with GAD, 18 subjects with PD, and 22 HC. All patients were recruited from the psychological outpatient clinic at Shanghai Mental Health Center (SMHC), China. Matched HC were recruited from local communities and Shanghai Jiaotong University. One expert clinician (CBL) confirmed the diagnoses based on the DSM-IV criteria for GAD and PD and the diagnoses are further checked by two research doctors (QH and LLZ) using Mini-International Neuropsychiatric Interview (MINI), Chinese version [Si et al., 2009]. Inclusion criteria were: (i) aged 18–60 years; (ii) medication free for at least 2 weeks; (iii) the scores of Hamilton Anxiety Scale (HAMA) [Hamilton, 1959] $\geq 14$, and the scores of Hamilton Depression Scale (HAMD) [Hamilton, 1960] $\leq 14$; (iv) have completed at least 6 years of primary education. Exclusion criteria were: (i) head trauma leading to loss of consciousness; (ii) severe somatic diseases, for example, cancer, heart failure, pneumonia; (iii) alcohol or substances abuse; (iv) neurological illnesses such as stroke and dementia; (v) contraindication to magnetic resonance (MR) scanning. HC were included in this study if they were 18–60 years of age and had at least 6 years of education. HC were also assessed using the MINI, and did not qualify for any psychiatric diagnoses. The same exclusion criteria were applied to HC. All subjects performed HAMA, HAMD, Spielberger State-Trait Anxiety Inventory (STAI) [Spielberger et al., 1970], Body Perception Questionnaire (BPQ) [Forges, 1993] before the fMRI scan. HAMA is a clinician-administered scale to assess anxiety symptoms which have two subscales, the psychic anxiety subscale and the somatic anxiety subscale. STAI is a self-administered assessment scale for the evaluation of severity of anxiety, containing trait anxiety and state anxiety. BPQ scale aims to capture body perception such as various bodily symptoms and sensations, which includes five factors: awareness, stress response, autonomic nervous system reactivity (ANSR), stress style, and disease history. Demographic and neuropsychological characteristics are listed in Table I. Our study was approved by the Research Ethics Committee at the SMHC of Shanghai Jiaotong University, China. All participants gave written informed consent before participating in this study.

**Image Acquisition**

All images were acquired on a 3.0-T SIMENS MAGNETOM TrioTim syngo MR B17 scanner equipped with a 12-
channel head coil at the Shanghai Key Laboratory of Magnetic Resonance of East China Normal University, Shanghai, China. Foam paddings were used to reduce head motion and earplugs were used to reduce scanner noise. Prior to the scan, the subjects were instructed to lie still with their eyes closed, not to fall asleep, to relax and to move as little as possible during scanning. A questionnaire was filled in prior to the scan, asking if the subjects had fallen asleep and open their eyes during the scanning. Foam paddings were used to reduce head motion (maximal motion between volumes in each direction, and rotation about each axis) of greater than 3 mm or rotation of greater than 3°. About 32 transverse slices (field of view = 240 × 240 mm, matrix = 64 × 64, slice thickness = 5 mm) resulting in a total of 157 volumes and a scan time of 314 s. No abnormalities were found upon inspection of the subjects’ structural images by a radiologist (JQL).

**fMRI Data Preprocessing**

Data quality control include: (i) any data affected by head motion (maximal motion between volumes in each direction, and rotation about each axis) of greater than 3 mm or rotation of greater than 3° was excluded; (ii) we discarded subjects with greater than 10% displaced frames from the analysis as it is likely that such high-level of movement would have had an influence on several volumes.

For the three datasets prior to preprocessing, the first 10 volumes were discarded to allow for scanner stabilization and the subjects’ adaptation to the environment. The fMRI data preprocessing was then conducted by SPM8 (http://www.fil.ion.ucl.ac.uk/spm) and a Data Processing Assistant for Resting-State fMRI (DPARSF). The remaining functional scans were first corrected for within-scan acquisition time differences between slices and then realigned to the middle volume to correct for inter-scan head motions. Subsequently, the functional scans were spatially normalized to a standard template (Montreal Neurological Institute) and resampled to 3 × 3 × 3 mm³. After normalization, BOLD signal of each voxel was firstly detrended to abandon linear trend and then passed through a band-pass filter (0.01–0.08 Hz) to reduce low-frequency drift and high-frequency physiological noise. Finally, nuisance covariates including head motion parameters, global mean signals, white matter signals and cerebrospinal signals were regressed out from the BOLD signals. A volume scrubbing movement correction [Liu et al., 2015] is carried out so that head-motion artifacts are not influencing observed effects. The mean framewise displacement (FD) was computed with FD threshold for displacement being 0.5. In addition to the frame corresponding to the displaced time point, one preceding and two succeeding time points were also deleted to reduce the “spill-over” effect from head movements. After data preprocessing, the time series were extracted in each ROI by averaging the signals of all voxels within that region.

In preprocessing we removed the global signal. Currently there is no consensus on the removal of global signal when computing FC. It has been shown that global signal removal can affect between-group analyses in schizophrenia but not in bi-polar disorder [Yang et al., 2014]. The global signal removal has been shown to reduce

<table>
<thead>
<tr>
<th>TABLE I. Demographic and neuropsychological characteristics</th>
</tr>
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<tbody>
<tr>
<td>Variables</td>
</tr>
<tr>
<td>Ages in years 39.95 (12.24)</td>
</tr>
<tr>
<td>Education in years 11.19 (3.31)</td>
</tr>
<tr>
<td>Sex (male/female) 13/7</td>
</tr>
<tr>
<td>HAMA-psychoic anxiety 13.60 (8.78)</td>
</tr>
<tr>
<td>HAMA-sonomic anxiety 8.83 (3.26)</td>
</tr>
<tr>
<td>STAI-trait 50.50 (12.0)</td>
</tr>
<tr>
<td>STAI-state 49.67 (15.30)</td>
</tr>
<tr>
<td>BPQ-awareness 99.44 (22.75)</td>
</tr>
<tr>
<td>BPQ-stress response 28.11 (9.76)</td>
</tr>
<tr>
<td>BPQ-ANSR 51.39 (14.38)</td>
</tr>
<tr>
<td>BPQ-stress style1 25.56 (3.97)</td>
</tr>
<tr>
<td>BPQ-stress style2 8.44 (2.53)</td>
</tr>
<tr>
<td>BPQ-diseases history 39.83 (12.61)</td>
</tr>
</tbody>
</table>

SD, standard deviation; HAMA, Hamilton Anxiety Scale; STAI, Spielberger State-Trait Anxiety Inventory; BPQ, Body Perception Questionnaire; ANSR, autonomic nervous system reactivity; GAD, generalized anxiety disorder; PD, panic disorder; HC, healthy control.
physiological noise, especially the variance due to movement related effects, thus improving the reliability of resting fMRI [Yan et al., 2013]. It also removes specific confounds from the data to facilitate the evaluation of neurophysiological relationships [Fox et al., 2009]. Currently there is no research on the global signal effect on anxiety disorders. The major argument against the removal of global signal is the introduction of spurious correlations. In our study the main interest is the difference between two groups in terms of the FC strength, irrespective of the sign of the FC, it is therefore important that both groups are treated identically. Note that the global signal in both groups are removed using identical approach, thus reducing the possibility of introducing spurious group differences. Moreover, though global signal removal can increase the frequency of pairwise negative correlation coefficients [Saad et al., 2012], we consider negative correlation only on relative terms rather than anticorrelations, which is consistent with Murphy et al. [2009]. It will be our future work to investigate the influence of global signal in whole-brain FC analysis.

For all three datasets the automated anatomical labeling atlas (AAL) was used to partition the brain into 90 regions of interest (ROIs) (45 per hemisphere). The names of the ROIs and their corresponding abbreviations are listed in Supporting Information Table S1.

### Scaled Correlation Coefficient as a Reliable FC Metric and Its High Statistical Power

Pearson correlation, or cross-correlation, has been extensively used as a FC metric [Biswal et al., 1995; Greicius et al., 2003]. In this article we used another version of cross-correlation, that is, scaled correlation coefficient [Nikolic et al., 2012] as a FC metric, which evaluates correlation coefficient at certain frequency ranges of the signal. Scaled correlation has been used to investigate synchronization hubs in the visual cortex [Folias et al., 2013]. In the following we first introduce the definition of Scaled correlation. We then describe the procedure needed to verify that scaled correlation has more statistical power in two sample t-test than Pearson correlation.

Scaled correlation between two time series is defined as the average correlation coefficient computed across short segments (or windows, with length s) of the two signals. Each signal is divided into K non-overlapped segments K = round(T/s), where T and s are the length of the entire signal and segment, respectively. Scaled correlation (rₖ) across the entire signals then is: rₖ = 1/K ∑ₖ=1^K rₖ, where rₖ is the cross-correlation of the Kth segments.

Scaled correlation with segment length s generally removes the correlation between frequency components (in two signals) lower than 1/s. The useful frequency range of BOLD signal is 0.01–0.08 Hz, which roughly corresponds to a time window of s = 100 seconds (0.01 Hz) and 12 seconds (0.08 Hz). Considering that the BOLD signal is sampled every 2 seconds, 100 and 12 seconds thus correspond to 50 and 6 sampled points, respectively. Therefore we compute scaled correlation with the segment length s varying from 6 to 50 sampled points, that is, s = 6, 7, 8, 9, ..., 50.

The reason why we perform scaled correlation analysis at each possible segment length is that: (1) We want to cover the 0.01–0.08 Hz frequency range that is shown to carry useful information in fMRI data. (2) To avoid arbitrary choice of segment length s. In practice, we used segment length varying from 6 to 30 (rather than 50) sampled points as the length of entire signal is relatively short and segment length larger than 30 sampled points will lead to too few segments to reliably estimate the scaled correlation. By adopting scaled correlation coefficient at each specific segment length s, we are able to pick out subtle FC changes in specific frequency bands that may not be identified under the scrutiny of traditional Pearson correlation.

Now we describe how to validate scaled correlation as a reliable FC metric by showing that it contains similar information to the traditional Pearson correlation, but has a high statistical power in t-test (i.e., more significant P value than that of Pearson correlation). We use a typical segment length (30 s) for demonstration. We perform t-test between scaled- and Pearson correlation for both the control and patient group for a given FC, and examine if scaled- and Pearson correlation differs significantly. We furthermore perform a correlation analysis between scaled- and Pearson correlation to see if they are correlated. Our hypothesis is that the mean of scaled correlation and Pearson correlation would not differ significantly in control and patient group. Furthermore, we check variance of scaled correlation in both control and patient group and compare to those obtained by Pearson correlation. If scaled correlation has smaller sample variance, then this could increase the statistical power in two-sample t-test.

### Statistical Analysis

For demographic and clinical characteristics, t-tests were analyzed using the Statistical Package for the Social Science version 19.0 (SPSS Inc., Chicago). These data were compared using t-tests for continuous variables, and chi-square test for categorical variables.

For FC analysis using scaled correlation, we perform two sample t-test for all connectivity of the brain network and identify those that show significant difference between patient and control groups (FDR, q = 0.05 and 0.1). Since scaled correlation is calculated with a given segment length s, to avoid arbitrary choice of segment length, we performed whole brain FC analysis using scaled correlation for each segment length s (ranging from 6 to 30 sampled-points, as was explained above). We then average the result obtained under each segment length, that is, we count the average number of significantly changed FC (denoted by m), and pick top m FC that appears most
frequently in the whole ensemble of identified FC under each segment length. This way, we are expected to obtain more stable FC changes. To evaluate the association between altered FC and symptom severity, we applied cross-correlation analysis \((P < 0.05)\).

**RESULTS**

**Demographic and Clinical Characteristics**

As is shown in Table I, both patients’ groups and HC group did not differ in age, gender and years of education. Both patients’ groups rated higher than HC on the two items (psychic anxiety and somatic anxiety) of the HAMA, the state and trait scales of the STAI, and the six subscales within BPQ (Table I). There was no statistically significant difference between GAD and PD subjects on all those scales mentioned above. On the HAMA-somatic anxiety subscale there was a trend for higher score in PD patients than in GAD, with the relatively small \(P\) value 0.084.

**FC Alterations in GAD and PD**

As can be seen in Figure 1a,b and Table II, totally 20 abnormal FC were found for GAD, the most significant abnormality in FC is seen between hippocampus/parahippocampus and fusiform gyrus (increased positive connectivity), compared with HC. Besides, disrupted FC also involve connectivities from precentral gyrus to visual areas such as lingual, calcarine and cuneus (decreased connectivity when compared with HC), as well as to inferior parietal (increased connectivity when compared with HC). FC from postcentral gyrus to cuneus and lingual gyrus are decreased and the connectivities from thalamus to posterior cingulate gyrus (PCC)/ACC are also decreased in GAD. Moreover, FC between middle frontal gyrus and angular gyrus is decreased and FC between supramarginal gyrus and precuneus is increased, in GAD compared with HC.

For PD, the FC of postcentral gyrus with thalamus are the most significant, which are increased in patients when compared with HC. Moreover, increased FC also involves the right ACC and the superior temporal gyrus, the right precentral gyrus and the thalamus. The FC from the left superior occipital gyrus to the medial temporal lobe is decreased in PD patients (see Fig. 1c and Table III).

**Validation of Scaled Correlation as a More Powerful FC Metric**

We choose the most significantly altered FC in GAD and PD (identified by scaled correlation, see Figs. 2 and 3) for demonstration. First we note that the mean FC obtained from scaled-correlation does not differ significantly from that obtained from scaled-correlation by Pearson-correlation for both control and patient group (including GAD and PD), see Supporting Information Tables S2 and S4. The mean FC obtained from that Pearson-correlation in fact show high level correlation (Supporting Information Tables S2 and S4). This indicates that scaled-correlation and Pearson-correlation bear similar information. However, the variance of the FC distribution
from scaled-correlation is much smaller than that of Pearson-correlation in both control and patient group, see Supporting Information Tables S3 and S5. According to the definition of t-value in two sample t-test in identifying significant changes,

\[
t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{\sigma_1^2}{n_1} + \frac{\sigma_2^2}{n_2}}}
\]

in which \( \bar{x}_1 \) and \( \bar{x}_2 \), and \( \sigma_1 \) and \( \sigma_2 \) are mean and standard deviation of two independent random variables \( x_1 \) and \( x_2 \) (i.e., the FC of control and patient group). The t value is proportional to the difference of the mean of \( x_1 \) and \( x_2 \), but is inversely proportional to the sum of variance of \( x_1 \) and \( x_2 \). As is shown in Supporting Information Tables S2–S5, the mean of scaled correlation as FC does not differ significantly with that of Pearson correlation, but the variance of scaled correlation is smaller, therefore the t value of t-test obtained from scaled correlation is larger than that derived from Pearson correlation, and that the difference between two groups are more significant. This is why scaled correlation has higher statistical power and why we can identify significantly changed FC in GAD/PD group by scaled correlation, but not Pearson correlation.

In fact the top functional connectivities identified from two sample t-test using Pearson correlation, though not significant (i.e., cannot survive correction), overlapped much with those obtained by scaled correlation, see Table IV. For

### TABLE II. Significantly altered functional connectivity by comparing GAD patients with matched controls (FDR \( q = 0.1 \); “\*” represent the FC that also survive FDR \( q = 0.05 \) correction)

<table>
<thead>
<tr>
<th>Functional connectivity</th>
<th>Increased†</th>
<th>Decreased‡</th>
<th>GAD (mean)</th>
<th>Control (mean)</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precentral gyrus (L)—Inferior parietal (R)</td>
<td>↑</td>
<td></td>
<td>0.1</td>
<td>−0.215</td>
<td>0.00009</td>
</tr>
<tr>
<td>ParaHippocampal gyrus (L)—Fusiform gyrus (L)</td>
<td>↑</td>
<td></td>
<td>0.422</td>
<td>0.171</td>
<td>0.00022</td>
</tr>
<tr>
<td>*Posterior cingulate gyrus (L)—Thalamus (L)</td>
<td>↓</td>
<td></td>
<td>0.067</td>
<td>0.27</td>
<td>0.00025</td>
</tr>
<tr>
<td>Hippocampus (R)—Fusiform gyrus (R)</td>
<td>↑</td>
<td></td>
<td>0.345</td>
<td>0.083</td>
<td>0.00028</td>
</tr>
<tr>
<td>*Precentral gyrus (L)—Lingual gyrus (L)</td>
<td>↓</td>
<td></td>
<td>−0.212</td>
<td>0.094</td>
<td>0.00028</td>
</tr>
<tr>
<td>*ParaHippocampal gyrus (R)—Fusiform gyrus (L)</td>
<td>↑</td>
<td></td>
<td>0.408</td>
<td>0.199</td>
<td>0.0003</td>
</tr>
<tr>
<td>Middle frontal gyrus (L)—Angular gyrus (R)</td>
<td>↑</td>
<td></td>
<td>0.187</td>
<td>0.431</td>
<td>0.00034</td>
</tr>
<tr>
<td>Precentral gyrus (R)—Cuneus (L)</td>
<td>↓</td>
<td></td>
<td>−0.15</td>
<td>0.128</td>
<td>0.00041</td>
</tr>
<tr>
<td>*Posterior cingulate gyrus (R)—Thalamus (L)</td>
<td>↑</td>
<td></td>
<td>0.09</td>
<td>0.321</td>
<td>0.00043</td>
</tr>
<tr>
<td>Cuneus (L)—Postcentral gyrus (R)</td>
<td>↑</td>
<td></td>
<td>0.12</td>
<td>0.176</td>
<td>0.00048</td>
</tr>
<tr>
<td>Precentral gyrus (L)—Calcarine cortex (L)</td>
<td>↑</td>
<td></td>
<td>−0.222</td>
<td>0.07</td>
<td>0.00055</td>
</tr>
<tr>
<td>SupraMarginal gyrus (L)—Precuneus (L)</td>
<td>↑</td>
<td></td>
<td>−0.003</td>
<td>−0.222</td>
<td>0.00056</td>
</tr>
<tr>
<td>Precentral gyrus (L)—Lingual gyrus (R)</td>
<td>↑</td>
<td></td>
<td>−0.206</td>
<td>0.048</td>
<td>0.00071</td>
</tr>
<tr>
<td>Precentral gyrus (L)—Cuneus (L)</td>
<td>↑</td>
<td></td>
<td>−0.266</td>
<td>−0.013</td>
<td>0.00071</td>
</tr>
<tr>
<td>Precentral gyrus (L)—Inferior parietal (L)</td>
<td>↑</td>
<td></td>
<td>0.337</td>
<td>0.132</td>
<td>0.00072</td>
</tr>
<tr>
<td>Precentral gyrus (R)—Lingual gyrus (L)</td>
<td>↑</td>
<td></td>
<td>0.044</td>
<td>0.219</td>
<td>0.00075</td>
</tr>
<tr>
<td>Cuneus (L)—Postcentral gyrus (L)</td>
<td>↑</td>
<td></td>
<td>−0.16</td>
<td>0.148</td>
<td>0.00079</td>
</tr>
<tr>
<td>Anterior cingulate gyrus (R)—Temporal pole (superior) (R)</td>
<td>↑</td>
<td></td>
<td>0.092</td>
<td>0.357</td>
<td>0.00086</td>
</tr>
<tr>
<td>Lingual gyrus (R)—Postcentral gyrus (L)</td>
<td>↑</td>
<td></td>
<td>−0.137</td>
<td>0.16</td>
<td>0.00092</td>
</tr>
<tr>
<td>Lingual gyrus (L)—Postcentral gyrus (L)</td>
<td>↑</td>
<td></td>
<td>−0.112</td>
<td>0.2</td>
<td>0.00093</td>
</tr>
</tbody>
</table>

Upward arrow indicates increased FC in patient group with respective to matched controls, and downward arrow indicates the opposite.

R, right. L, left. Names and abbreviations of brain regions can be referred to Supporting Information Table S1.

### TABLE III. Significantly altered functional connectivity by comparing PD patients with matched controls (FDR \( q = 0.1 \); “\*” represent the FC that also survive FDR \( q = 0.05 \) correction)

<table>
<thead>
<tr>
<th>Functional connectivity</th>
<th>Increased†</th>
<th>Decreased‡</th>
<th>PD (mean)</th>
<th>Control (mean)</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Postcentral gyrus (R)—Thalamus (L)</td>
<td>↑</td>
<td></td>
<td>−0.008</td>
<td>−0.335</td>
<td>0.00007</td>
</tr>
<tr>
<td>*Anterior cingulate gyrus (R)—Temporal pole (superior) (R)</td>
<td>↑</td>
<td></td>
<td>0.269</td>
<td>−0.056</td>
<td>0.00009</td>
</tr>
<tr>
<td>*Superior occipital gyrus (L)—Temporal Pole (middle) (R)</td>
<td>↓</td>
<td></td>
<td>−0.309</td>
<td>−0.009</td>
<td>0.0001</td>
</tr>
<tr>
<td>Postcentral gyrus (L)—Thalamus (R)</td>
<td>↑</td>
<td></td>
<td>0.05</td>
<td>−0.297</td>
<td>0.00015</td>
</tr>
<tr>
<td>Precentral gyrus (R)—Thalamus (L)</td>
<td>↑</td>
<td></td>
<td>0.096</td>
<td>−0.266</td>
<td>0.00018</td>
</tr>
<tr>
<td>Postcentral gyrus (L)—Thalamus (L)</td>
<td>↑</td>
<td></td>
<td>0.079</td>
<td>−0.27</td>
<td>0.00025</td>
</tr>
<tr>
<td>Precentral gyrus (R)—Thalamus (R)</td>
<td>↑</td>
<td></td>
<td>0.105</td>
<td>−0.244</td>
<td>0.00033</td>
</tr>
</tbody>
</table>
GAD, the top 20 FC obtained by Pearson correlation has 10 that are overlapped with those from scaled correlation. For PD, the top 7 FC obtained by Pearson correlation are completely overlapped with those from scaled correlation. These results indicate the scaled correlation is a reliable but more powerful FC metric in two sample t-test.

**Figure 2.** Distribution of scaled correlation (a) and Pearson correlation (b) for control and GAD group (for the first FC in Table II). As can be seen, the distribution of scaled correlation and Pearson correlation has similar mean (for both control and GAD group, with \( P > 0.05 \), see also Supporting Information Table S2), while the variance of the former is smaller than that of the latter (see Supporting Information Table S3).

**Figure 3.** Distribution of scaled correlation (a) and Pearson correlation (b) for control and PD group (for the first FC in Table III). As can be seen, the distribution of scaled correlation and Pearson correlation has similar mean (for both control and PD group, with \( P > 0.05 \), see Supporting Information Table S4), while the variance of the former is smaller than that of the latter (see Table S5, Supporting Information).
Correlation between FC Alteration and Symptom Scores

For GAD, the disrupted connectivity between hippocampus/parahippocampus and fusiform gyrus correlated to BPQ subscores (including subscores for awareness, stress response, autonomic nervous system reactivity and disease history) in positive way, while connectivity between ACC and thalamus correlated negatively with HAMA-somatic/psychic anxiety and STAI-trait. For PD, the altered connectivity in GAD HC (mean) GAD (mean) P (Pearson-corr)

<table>
<thead>
<tr>
<th>Functional connectivity</th>
<th>Score type</th>
<th>P value</th>
<th>Corr. coef.</th>
</tr>
</thead>
<tbody>
<tr>
<td>ParaHippocampal gyrus (L)—Fusiform gyrus (L)</td>
<td>BPQ-awareness</td>
<td>0.0081</td>
<td>0.487</td>
</tr>
<tr>
<td>ParaHippocampal gyrus (L)—Fusiform gyrus (L)</td>
<td>BPQ-stress response</td>
<td>0.0022</td>
<td>0.5577</td>
</tr>
<tr>
<td>ParaHippocampal gyrus (L)—Fusiform gyrus (L)</td>
<td>BPQ-ANSR</td>
<td>0.002</td>
<td>0.563</td>
</tr>
<tr>
<td>ParaHippocampal gyrus (L)—Fusiform gyrus (L)</td>
<td>BPQ-diseases history</td>
<td>0.0103</td>
<td>0.472</td>
</tr>
<tr>
<td>Posterior cingulate gyrus (L)—Fusiform gyrus (L)</td>
<td>BPQ-diseases history</td>
<td>0.0469</td>
<td>0.3671</td>
</tr>
<tr>
<td>Cuneus (L)—Postcentral gyrus (R)</td>
<td>BPQ-diseases history</td>
<td>0.0314</td>
<td>0.3971</td>
</tr>
<tr>
<td>Cuneus (L)—Postcentral gyrus (R)</td>
<td>STAI-state</td>
<td>0.0132</td>
<td>0.4536</td>
</tr>
<tr>
<td>Precentral gyrus (L)—Calcarine cortex (L)</td>
<td>STAI-trait</td>
<td>0.0389</td>
<td>0.3792</td>
</tr>
<tr>
<td>Anterior cingulate gyrus (R)—Thalamus (R)</td>
<td>STAI-trait</td>
<td>0.0993</td>
<td>0.6101</td>
</tr>
<tr>
<td>Anterior cingulate gyrus (R)—Thalamus (R)</td>
<td>HAMA-somatic anxiety</td>
<td>0.0261</td>
<td>0.4227</td>
</tr>
<tr>
<td>Anterior cingulate gyrus (R)—Thalamus (R)</td>
<td>HAMA-psychic anxiety</td>
<td>0.0399</td>
<td>0.5024</td>
</tr>
</tbody>
</table>
connectivity between post/precentral and thalamus is found to be positively related to STAI-state and BPQ subscores (subscals for awareness and stress response) (see Tables V and VI).

**DISCUSSION**

To the best of our knowledge, our study is the first whole-brain resting state FC analysis involving both GAD and PD. This allows the common and disease-specific functional abnormalities to be systematically investigated. Our results, therefore, are expected to shed new lights onto the distinct neural mechanisms of these two major anxiety disorders. Note that no significant FC changes were identified using Pearson correlation for both GAD and PD group. Therefore, we described the two separate \( t \)-test (GAD vs. HC, PD vs. HC) here.

**Differences in Resting FC Between GAD and PD**

As is shown in Tables II and III, much of the disrupted FC in GAD and PD shows regional and psychopathological specificity. For GAD, the aberrant connectivity of fusiform gyrus with hippocampus/parahippocampus are among the most significantly changed FC in GAD. Hippocampus and parahippocampus are parts of limbic system that are implicated in memory [Maren and Holt, 2000; Squire, 1992] and emotion [Alvarez et al., 2008; Chen and Etkin, 2013]. The reduction of hippocampus volume has been found in patients with GAD [Abdallah et al., 2013; Hettema et al., 2012; Moon et al., 2014] while the parahippocampus has also been implicated in anxiety disorders [Hattingh et al., 2012; Lorberbaum et al., 2004].

The fusiform gyrus is part of the temporal lobe and occipital lobe that has been suggested to play an important role in face, body, and word recognition [Grill-Spector and Malach, 2004; Haxby et al., 2000, 2002; Kanwisher et al., 1997; Martin, 2007]. Abnormal activities in the fusiform gyrus in response to emotional faces have been reported in different anxiety disorders [Etkin and Wager, 2007; Gentili et al., 2008; Schultz et al., 2003; Syal et al., 2012].

The above findings of fusiform gyrus suggest that it might be a core brain region in visual and emotion processing in anxiety disorders. Both the visual cortex and hippocampus are involved in the processing of fear generalization [Lissek, 2012; Lissek et al., 2013a; Xu and Sudhof, 2013] which may contribute importantly to the psychopathology of GAD [Lissek et al., 2013b]. This is supported by our observation of the increased hippocampal-fusiform gyrus FC correlating with BPQ subscores in GAD, since BPQ were used to measure multiple symptom areas [Stromback et al., 2015].

In addition, cuneus-postcentral gyrus connection and precentral gyrus-calcarine cortex connection are abnormal in GAD, and correlate with severity of symptoms, see Table V. The cuneus is associated with contextual self-descriptions [Chiao et al., 2009]. The postcentral gyrus and precentral gyrus have been associated with interoception processing [Critchley et al., 2004; Northoff, 2013; Pollatos et al., 2007].

Calcarine is reported to be associated with working memory in GAD [Moon and Jeong, 2015]. These abnormal connections may reflect a widely disturbed network in GAD.

Finally, three functional connections are associated with PCC/ACC-thalamus pathway. PCC is the backmost part of the cingulated cortex and a main component of DMN which is associated with functions including emotional processing and social cognition [Broyd et al., 2009]. The abnormal FC within this area suggests that process relying on the DMN is affected in GAD. ACC is the frontal part of the cingulated cortex and participates in mediating visceromotor activity [Devinsky et al., 1995]. It is a main component of the interoceptive network [Khalsa et al., 2009]. The major role of the thalamus is to relay the information of sensation, motor signals, and spatial sense to the cerebral cortex [Sherman, 2007]. Given our observation that decreased functional connections between ACC and thalamus that was negatively correlated with HAMA in GAD patient therefore may cause some symptoms involve somatic disturbances like rapid heart rate, lower skin conductance, and difficulty breathing [Hoehn-Saric et al., 2004]. Over-generalization might be the main abnormal mechanism underlying GAD patients may answer why these patients experience less intense heartbeat perception than patients with PD.

For PD, the connectivity between postcentral gyrus and thalamus account for most of the significant alterations. Postcentral gyrus is known as the somatosensory cortex with function of receiving, integrating and interpreting most of the sensory information of the human body [Northoff, 2013]. Sensory information is carried to the brain by neural pathways to the thalamus, which project to the somatosensory cortex [Nelson and Chen, 2008].

Thalamus plays a crucial role in gating sensory responses [Saalmann and Kastner, 2011]. The increased FC from

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**TABLE VI. Correlation between altered functional connectivity and symptom scores for PD patients**

<table>
<thead>
<tr>
<th>Functional connectivity</th>
<th>Score type</th>
<th>( P ) value</th>
<th>Corr. coef.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postcentral gyrus (L)—Thalamus (R)</td>
<td>STAI-state</td>
<td>0.046</td>
<td>0.3399</td>
</tr>
<tr>
<td>Precentral gyrus (R)—Thalamus (L)</td>
<td>STAI-state</td>
<td>0.0366</td>
<td>0.4176</td>
</tr>
<tr>
<td>Precentral gyrus (R)—Thalamus (R)</td>
<td>STAI-state</td>
<td>0.0366</td>
<td>0.4176</td>
</tr>
<tr>
<td>*Superior occipital gyrus (L)—Temporal Pole (middle) (R)</td>
<td>HAMA-psychic anxiety</td>
<td>0.0412</td>
<td>-0.515</td>
</tr>
<tr>
<td>Postcentral gyrus (L)—Thalamus (L)</td>
<td>BPQ-awareness</td>
<td>0.076</td>
<td>0.3096</td>
</tr>
<tr>
<td>Postcentral gyrus (L)—Thalamus (L)</td>
<td>BPQ-stress response</td>
<td>0.082</td>
<td>0.3076</td>
</tr>
</tbody>
</table>
thalamus to somatosensory cortex found in PD patients therefore may cause abnormally high interoceptive sensiti-
vity and somatosensory stimulus processing, which underlies the typical symptoms of PD, such as the extreme feel-
ing of heartbeat. This finding is consistent with that the
somatosensory cortex is the critical substrate for interoce-
ptive awareness [Khalasa et al., 2009] and the thalamus has
been shown to be strongly involved in interoceptive pro-
cessing [Cho et al., 2012; Craig, 2003; Craig and Zhang,
2006; Pollatos et al., 2007; Rieck et al., 2004]. Interestingly,
thalamus does not only play a role in interoceptive pro-
cessing, but may also be crucially involved in the fear cir-
cuit underlying the pathogenesis of PD [Gorman et al.,
2000]. Considerable neuroimaging studies investigating
functional and structural brain connectivity in PD show
abnormalities in thalamus [Asami et al., 2009; Gorman
et al., 2000; Lai and Wu, 2012, 2013; Ohrrmann et al., 2010;
Winternann et al., 2013; Zhang et al., 2011]. Phobic
patients also have increased activities in the thalamus dur-
ing panic attacks [Caseras et al., 2010]. Therefore, the thal-
amus plays an important role in the pathogenesis of PD,
possibly via the mediation of interoceptive processes.
The postcentral gyrus showed increased resting-state FC with
left dorsal ACC for salience network in PD patients [de
Carvalho et al., 2010; Pannekoek et al., 2013], with the
finding possibly suggest that the processing of somatosen-
sory information and self-awareness is disturbed in PD.
The postcentral gyrus also showed abnormal activities
during a motor activation paradigm in female subjects
with PD which suggest that the postcentral gyrus is impli-
cated in the pathological mechanism of PD [Marchand
et al., 2009]. In summary, combined with the positive cor-
relation between strengthened connections and the severe-
ity of panic anxiety symptoms (Table VI), increased
postcentral gyrus-thalamus connectivity could be an early
biomarker in PD patients.

Limitations

Our data have to be interpreted with caution due to sev-
eral limitations. The main limitation of this study is the
small number of sample subjects, which deteriorates the
statistical power and makes it susceptible to type II error.
However, we have used scaled correlation as FC, which
we demonstrate to be a reliable (compared with Pearson
correlation) but statistically more powerful metric. We also
note that the identified FC in both GAD and PD group
demonstrate symmetric patterns. In GAD, significant
changes involve connectivity of left cuneus to bilateral
pre/postcentral gyrus, bilateral lingual gyrus to bilateral
precentral gyrus, and left thalamus to bilateral post-
cingulate. In PD, changes include FC of bilateral thalamus
to bilateral postcentral gyrus. These symmetric FC changes
add to the reliability of the results, as the probability for
two symmetric connectivity to change significantly in a
simultaneous manner would be low if they are true nega-
tive. Secondly, the fMRI data was acquired using the
parameters: TR = 2 s, slices = 30, bandpass filtering in the
range 0.01 < f < 0.08 Hz. Under such condition of the
acquisition, cardiac, and respiratory fluctuations may still
reduce the specificity of low frequency fluctuations to
functional connected regions [Lowe et al., 1998]. Finally,
our resting-state fMRI data were acquired after the acquisi-
tion of an anatomical scan and completion of four task-
related fMRI runs. The acquisition protocol could poten-
tially have influence on resting-state connectivity.

CONCLUSIONS

Taken together, our results suggest disease-specific FC
abnormalities responsible for GAD and PD patients. GAD
is more associated with the fusiform gyrus and hippocam-
pus/parahippocampus pathways suggesting the mechani-
sm of fear generalization. PD is rather linked to
interoceptive pathways involving somatosensory cortex and
thalamus. Future studies are needed to explore the effect
of anti-anxiety medications on resting state activity and its
regionally- and psychopathologically specific changes in
GAD and PD. This in turn may not only be relevant for
diagnosis but also or selection of differential treatment
strategies in GAD and PD.

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