

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* 37:1459–1473, 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 viscerosensation (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, precentral 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 amygdala [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 cingulate cortex (ACC), frontal and

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 connective 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-naïve) 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; Strawn et al., 2012; Wittmann et al., 2011] that mostly focused on ROIs defined 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] ≥ 14 , and the scores of Hamilton Depression Scale (HAMD) [Hamilton, 1960] ≤ 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) [Porges, 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-

TABLE I. Demographic and neuropsychological characteristics

Variables	GAD	PD	HC	Groups comparisons		
	<i>n</i> = 21 Mean (SD)	<i>n</i> = 18 Mean (SD)	<i>n</i> = 22 Mean (SD)	GAD vs. HC <i>P</i> value	PD vs. HC <i>P</i> value	GAD vs. PD <i>P</i> value
Ages in years	39.95 (12.24)	37.17 (11.31)	38.05 (10.32)	0.593	0.801	0.473
Education in years	11.19 (3.31)	10.89 (2.68)	12.50 (2.59)	0.142	0.084	0.746
Sex (male/female)	13/7	12/6	14/8	0.927	0.842	0.914
HAMA-psychic anxiety	13.60 (8.78)	10.33 (4.01)	0.81 (1.44)	0	0	0.182
HAMA-somatic anxiety	8.83 (3.26)	13.33 (11.89)	0.14 (0.36)	0	0	0.084
STAI-trait	50.50 (12.0)	54.25 (8.00)	39.35 (8.33)	0.002	0	0.298
STAI-state	49.67 (15.30)	50.00 (10.78)	35.17 (9.09)	0.001	0	0.943
BPQ-awareness	99.44 (22.75)	110.06 (24.97)	75.30 (20.40)	0.001	0	0.197
BPQ-stress response	28.11 (9.76)	30.94 (8.60)	16.60 (5.77)	0	0	0.37
BPQ-ANSR	51.39 (14.38)	53.59 (13.92)	38.40 (8.01)	0.001	0	0.649
BPQ-stress style1	25.56 (3.97)	24.47 (4.12)	20.6 (4.42)	0.001	0.01	0.434
BPQ-stress style2	8.44 (2.53)	8.82 (2.63)	5.65 (2.16)	0.001	0	0.349
BPQ-diseases history	39.83 (12.61)	36.12 (7.02)	29.65 (3.50)	0.001	0.001	0.862

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.

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 after the MRI indicated that whether or not the subjects had fallen asleep and open their eyes during the scanning. The data with abnormal head move would be discarded. High-resolution T1-weighted anatomical images were acquired for registration in the sagittal orientation using a magnetization-prepared rapid gradient-echo sequence (repetition time = 1900 ms, echo time = 2.46 ms, flip angle = 9°, 32 transverse slices, field of view = 240 × 240 mm, matrix = 256 × 256, slice thickness = 1 mm). Resting-state functional MRI data were acquired using a single-shot, gradient-recalled echo planar imaging sequence (repetition time = 2000 ms, echo time = 25 ms, flip angle = 90°). 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 discard 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

physiological noise, especially the variance due to movement related effects, thus improving the reliability of resting fMRI [Yan et al., 2013]. It also remove 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 $K = \text{round}(T/s)$, where T and s are the length of the entire signal and segment, respectively. Scaled correlation (r_s) across the entire signals then is: $r_s = \frac{1}{K} \sum_{k=1}^K r_k$, where r_k is the cross-correlation of the K th 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 sig-

nal 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, $s = 6, 7, 8, 9, \dots, 50$.

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 further more 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

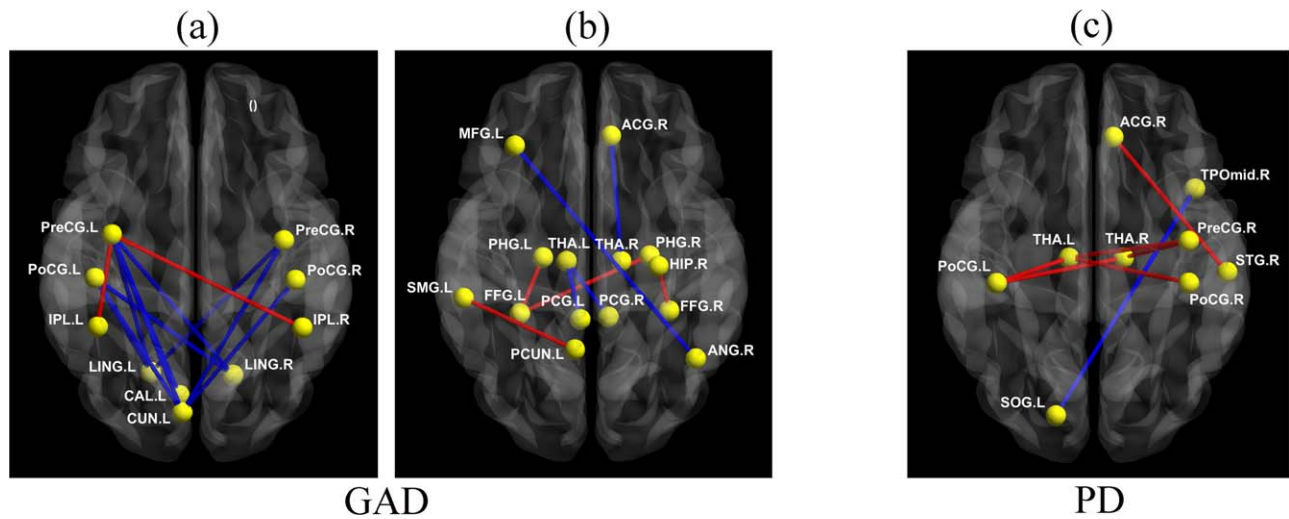


Figure 1.

Altered functional connectivity of GAD (a, b) and PD (c). Abnormal FC in GAD were divided into two graphs (a, b) in order to display more clearly. Red line indicates increased FC in patient group with respective to matched controls, and blue line indicates the opposite. (Note that names and abbreviations of brain regions can be referred to Table S1, Supporting Information).

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

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)

Functional connectivity	Increased↑ Decreased↓	GAD (mean)	Control (mean)	P value
*Precentral gyrus (L)—Inferior parietal (R)	↑	0.1	-0.215	0.00009
ParaHippocampal gyrus (L)—Fusiform gyrus (L)	↑	0.422	0.171	0.00022
*Posterior cingulate gyrus (L)—Thalamus (L)	↓	0.067	0.27	0.00025
Hippocampus (R)—Fusiform gyrus (R)	↑	0.345	0.083	0.00028
*Precentral gyrus (L)—Lingual gyrus (L)	↓	-0.212	0.094	0.00028
*ParaHippocampal gyrus (R)—Fusiform gyrus (L)	↑	0.408	0.199	0.0003
Middle frontal gyrus (L)—Angular gyrus (R)	↓	0.187	0.431	0.00034
Precentral gyrus (R)—Cuneus (L)	↓	-0.15	0.128	0.00041
*Posterior cingulate gyrus (R)—Thalamus (L)	↓	0.09	0.321	0.00043
Cuneus (L)—Postcentral gyrus (R)	↓	-0.12	0.176	0.00048
Precentral gyrus (L)—Calcarine cortex (L)	↓	-0.222	0.07	0.00055
SupraMarginal gyrus (L)—Precuneus (L)	↑	-0.003	-0.222	0.00056
Precentral gyrus (L)—Lingual gyrus (R)	↓	-0.206	0.048	0.00071
Precentral gyrus (L)—Cuneus (L)	↓	-0.266	-0.013	0.00071
Precentral gyrus (L)—Inferior parietal (L)	↑	0.397	0.132	0.00072
Precentral gyrus (R)—Lingual gyrus (L)	↓	-0.044	0.219	0.00075
Cuneus (L)—Postcentral gyrus (L)	↓	-0.16	0.148	0.00079
Anterior cingulate gyrus (R)—Thalamus (R)	↓	0.092	0.357	0.00086
Lingual gyrus (R)—Postcentral gyrus (L)	↓	-0.137	0.16	0.00092
Lingual gyrus (L)—Postcentral gyrus (L)	↓	-0.112	0.2	0.00093

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.

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,

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{\sigma_{x_1}^2 + \sigma_{x_2}^2}{n-1}}}$$

in which \bar{x}_1 and \bar{x}_2 , and σ_{x_1} and σ_{x_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 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)

Functional connectivity	Increased↑ Decreased↓	PD (mean)	Control (mean)	P value
*Postcentral gyrus (R)—Thalamus (L)	↑	-0.008	-0.335	0.00007
*Anterior cingulate gyrus (R)—Temporal pole (superior) (R)	↑	0.269	-0.056	0.00009
*Superior occipital gyrus (L)—Temporal Pole (middle) (R)	↓	-0.309	-0.009	0.0001
Postcentral gyrus (L)—Thalamus (R)	↑	0.05	-0.297	0.00015
Precentral gyrus (R)—Thalamus (L)	↑	0.096	-0.266	0.00018
Postcentral gyrus (L)—Thalamus (L)	↑	0.079	-0.27	0.00025
Precentral gyrus (R)—Thalamus (R)	↑	0.105	-0.244	0.00033

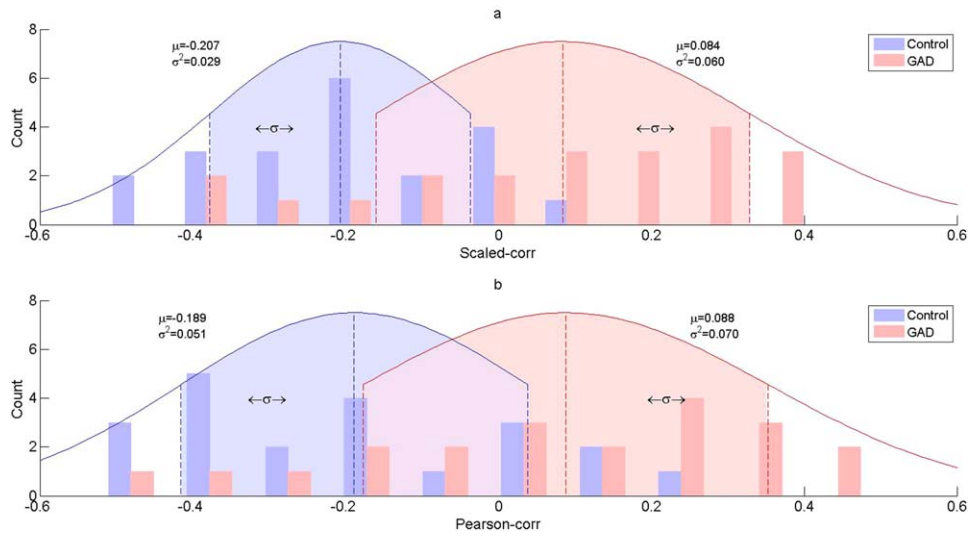


Figure 2.

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).

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 com-

pletely 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.

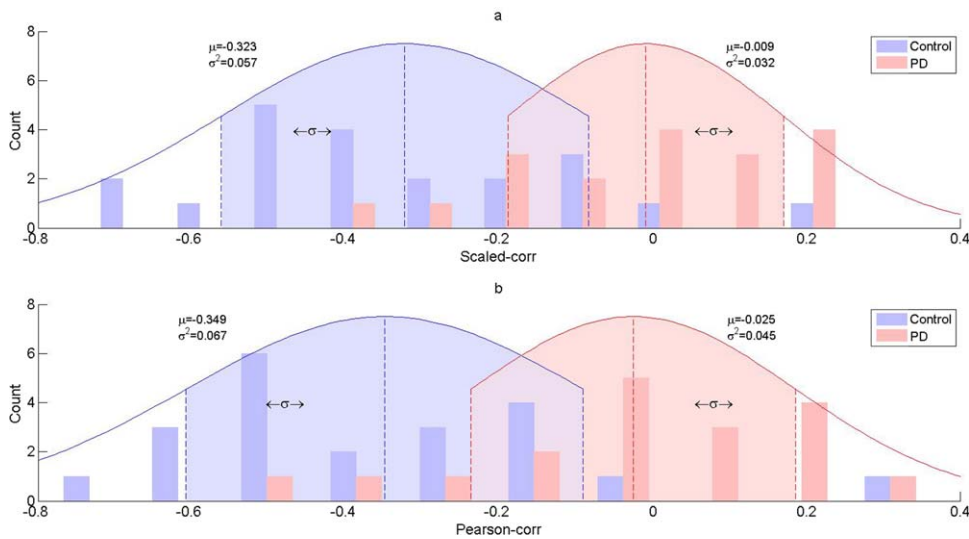


Figure 3.

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).

TABLE IV. Top links identified by Pearson correlation for GAD (a, top 20 links) and PD (b, top 7 links) group

Functional connectivity in GAD	HC (mean)	GAD (mean)	<i>P</i> (Pearson-corr)
(a) GAD			
*ParaHippocampal gyrus (L)—Fusiform gyrus (L)	0.1835	0.4525	0.000016
*Posterior cingulate gyrus (L)—Thalamus (L)	0.2830	0.0839	0.000097
Anterior cingulate gyrus (R)—Inferior parietal lobule (R)	0.1506	-0.1440	0.000126
* Middle frontal gyrus (L)—Angular gyrus (R)	0.4337	0.1795	0.000139
* Cuneus (L)—Postcentral gyrus (R)	0.1576	-0.1399	0.000200
*Anterior cingulate gyrus (R)—Thalamus (R)	0.3830	0.1053	0.000207
Anterior cingulate gyrus (L)— Inferior parietal lobule (R)	0.0252	-0.2545	0.000230
Supplementary motor area (R)—Angular gyrus (L)	-0.3682	-0.1777	0.000291
Precentral gyrus (R)—Middle temporal gyrus (L)	0.0681	-0.1899	0.000312
* Posterior cingulate gyrus (R)—Thalamus (L)	0.3334	0.1117	0.000320
Precentral gyrus (R)—Thalamus (R)	-0.2617	0.0170	0.000349
*Precentral gyrus (L)— Inferior parietal lobule (R)	-0.1888	0.0879	0.000391
*Precentral gyrus (R)—Cuneus (L)	0.1002	-0.1584	0.000432
Superior frontal gyrus (R)—Hippocampus (L)	-0.0095	-0.2302	0.000481
Cuneus (R)—Postcentral gyrus (R)	0.1955	-0.0723	0.000495
*Precentral gyrus (L)—Lingual gyrus (L)	0.0750	-0.1826	0.000579
*Precentral gyrus (L)—Cuneus (L)	-0.0425	-0.2610	0.000590
Postcentral gyrus (L)—Thalamus (R)	-0.3219	-0.0596	0.000621
Hippocampus (L)—Fusiform gyrus (L)	0.1438	0.3733	0.000652
Supplementary motor area (L)—Calcarine cortex (R)	-0.0451	-0.2558	0.000659
(b) PD			
*Superior occipital gyrus (L)—Temporal pole, middle (R)	-0.0058	-0.3159	0.000020
*Postcentral gyrus (L)—Thalamus (R)	-0.3219	0.0193	0.000039
*Precentral gyrus (R)—Thalamus (L)	-0.2761	0.1013	0.000060
*Postcentral gyrus (R)—Thalamus (L)	-0.3490	-0.0253	0.000073
*Postcentral gyrus (L)—Thalamus (L)	-0.2835	0.0552	0.000103
*Precentral gyrus (R)—Thalamus (R)	-0.2617	0.0904	0.000154
*Anterior cingulate gyrus (R)—Superior temporal gyrus (R)	-0.0564	0.2367	0.000208

Note that no significant FC changes were identified using Pearson correlation (FDR, $q = 0.05$) for both GAD and PD, and we listed the FC that changes most significantly in patient group (by their *P* value). The link marked with “*” are those identified by scaled correlation (see Table II for GAD and III for PD). As can be seen, for GAD, 10 of the top 20 links identified by Pearson correlation are found to change significantly using scaled correlation. For PD, the top 7 links by Pearson correlation are all identified by scaled correlation.

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

TABLE V. Correlation between altered functional connectivity and symptom scores for GAD patients

Functional connectivity	Score type	<i>P</i> value	Corr. coef.
ParaHippocampal gyrus (L)—Fusiform gyrus (L)	BPQ-awareness	0.0081	0.487
ParaHippocampal gyrus (L)—Fusiform gyrus (L)	BPQ-stress response	0.0022	0.5577
ParaHippocampal gyrus (L)—Fusiform gyrus (L)	BPQ-ANSR	0.002	0.563
ParaHippocampal gyrus (L)—Fusiform gyrus (L)	BPQ-diseases history	0.0103	0.472
Hippocampus (R)—Fusiform gyrus (R)	BPQ-diseases history	0.0469	0.3671
Cuneus (L)—Postcentral gyrus (R)	BPQ-diseases history	0.0314	-0.3971
Cuneus (L)—Postcentral gyrus (R)	STAI-state	0.0132	-0.4536
Precentral gyrus (L)—Calcarine cortex (L)	STAI-trait	0.0389	-0.3792
Anterior cingulate gyrus (R)—Thalamus (R)	STAI-trait	0.0093	-0.6101
Anterior cingulate gyrus (R)—Thalamus (R)	HAMA-somatic anxiety	0.0261	-0.4227
Anterior cingulate gyrus (R)—Thalamus (R)	HAMA-psychic anxiety	0.0399	-0.5024

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

Functional connectivity	Score type	<i>P</i> value	Corr. coef.
Postcentral gyrus (L)—Thalamus (R)	STAI-state	0.046	0.3399
Precentral gyrus (R)—Thalamus (L)	STAI-state	0.0366	0.4176
Precentral gyrus (R)—Thalamus (R)	STAI-state	0.0366	0.4176
*Superior occipital gyrus (L)—Temporal Pole (middle) (R)	HAMA-psychic anxiety	0.0412	−0.515
Postcentral gyrus (L)—Thalamus (L)	BPQ-awareness	0.076	0.3096
Postcentral gyrus (L)—Thalamus (L)	BPQ-stress response	0.082	0.3076

connectivity between post/precentral and thalamus is found to be positively related to STAI-state and BPQ subscores (subscales 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 cingulate 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 cingulate 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

thalamus to somatosensory cortex found in PD patients therefore may cause abnormally high interoceptive sensitivity and somatosensory stimulus processing, which underlies the typical symptoms of PD, such as the extreme feeling of heartbeat. This finding is consistent with that the somatosensory cortex is the critical substrate for interoceptive awareness [Khalsa et al., 2009] and the thalamus has been shown to be strongly involved in interoceptive processing [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 processing, but may also be crucially involved in the fear circuit 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; Ohrmann et al., 2010; Wintermann et al., 2013; Zhang et al., 2011]. Phobic patients also have increased activities in the thalamus during panic attacks [Caseras et al., 2010]. Therefore, the thalamus 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 somatosensory 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 implicated in the pathological mechanism of PD [Marchand et al., 2009]. In summary, combined with the positive correlation between strengthened connections and the severity 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 several 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-

tives. 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 acquisition of an anatomical scan and completion of four task-related fMRI runs. The acquisition protocol could potentially 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 hippocampus/parahippocampus pathways suggesting the mechanism 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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