

## Research paper

# Abnormal dynamic functional connectivity density in patients with generalized anxiety disorder



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## ABSTRACT

**Background:** Numerous studies have revealed the abnormal static functional connectivity (FC) among different brain regions in patients with generalized anxiety disorder (GAD). However, little is known about the dynamic changes of FC in patients with GAD.

**Methods:** This study investigated the whole-brain dynamic changes of FC in patients with GAD by combining global FC density (FCD) and sliding window correlation analyses. The standard deviation of dynamic FCD (dFCD) was calculated to evaluate its temporal variability along time. Support vector regression was then employed to predict the symptom severity of patients based on abnormal dynamic connectivity patterns.

**Results:** The abnormal dFCD variability between 81 GAD patients and 80 healthy controls showed that the patients had higher dFCD variability in the bilateral dorsomedial prefrontal cortex (dmPFC) and left hippocampus while lower dFCD variability in the right postcentral gyrus. The abnormal dFCD variability of the left dmPFC is an important feature for anxiety prediction.

**Limitations:** The selection of sliding window length remains controversial, and most of our patients have been treated with medications. Future studies are expected to rule out the potential confounding effects from applying different parameters of the sliding window and recruiting large samples of medication-free patients.

**Conclusion:** The altered patterns of time-varying brain connectivity in the frontolimbic and sensorimotor areas may reflect abnormal dynamic neural communication between these regions and other regions of the brain, which may deepen our understanding of the disease.

## 1. Introduction

Generalized anxiety disorder (GAD) is a prevalent psychiatric disease accompanied by chronic, persistent, excessive, and uncontrollable worry (Fonzo and Etkin, 2016; Makovac et al., 2016; Schienle et al., 2011) and unreasonable fear among a variety of aspects in daily life (Strawn et al., 2012; Tyrer and Baldwin, 2006). GAD is a common anxiety disorder subtype, which has the lowest remission rate after treatment compared with other anxiety disorders (Buff et al., 2016; Kinney et al., 2017). Patients with GAD often become easily fatigued, restless and irritable and have increased muscle tension and difficulty in concentrating and sleeping (DeMartini et al., 2019). Although, numerous neuroimaging studies have been performed to investigate the pathological basis of the disease, the underlying mechanisms remain poorly characterized.

A significant feature of GAD is emotion dysregulation (Blair et al., 2012; Etkin et al., 2010; Mochcoyitch et al., 2014; Palm et al., 2011), which is characterized by emotional hyperarousal, poor understanding of emotions, negative attitudes about emotions, and maladaptive emotion management and regulation (Behar et al., 2009). The hyper-response to negative emotion has been repeatedly reported in patients with GAD, manifesting itself as over-activation in the limbic system (Fonzo et al., 2015; McClure et al., 2007; Monk et al., 2008; Moon and Jeong, 2015; Park et al., 2016) and is often accompanied by hypo-activation in the prefrontal cortex (Monk et al., 2008; Moon and Jeong, 2015; Palm et al., 2011; Via et al., 2018; Wang et al., 2018a), which is associated with emotional dysregulation. Furthermore, the abnormal functional connectivity (FC) of the default mode network (DMN) (Diefenbach et al., 2019; Rabany et al., 2017; Roy et al., 2013), frontal-parietal network and salience network (Etkin et al., 2009;

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Rabany et al., 2017) is implicated in the impairment of emotional regulation in patients with GAD. These findings are consistent with the cognitive models of GAD, which propose that GAD patients show obvious impairments in cognitive control (Hirsch and Mathews, 2012), including negative attention bias (Fonzo et al., 2015; McClure et al., 2007; Monk et al., 2008), inhibition impairments (Hallion et al., 2017) and disrupted performance in working memory (Moon and Jeong, 2015; Park et al., 2016). The brain regions associated with these impairments are widely distributed across the frontal-parietal cortices, which belong to the executive system (Cui et al., 2014). These regions include the ventral prefrontal cortex, dorsolateral prefrontal cortex, superior parietal gyrus and postcentral gyrus.

The abovementioned studies were based on the assumption that brain activity is relatively stable during functional magnetic resonance imaging (fMRI) scanning. However, brain activity is dynamic and is accompanied by ongoing change over time (Calhoun et al., 2014; Hutchison et al., 2013; Li et al., 2018b; Liao et al., 2019; Yao et al., 2017), which may result in dynamic connectivity among discrete brain regions. The dynamic connectivity alterations might be potential biomarkers of specific diseases (Jones et al., 2012; Sakoglu et al., 2010) and be useful for searching additional abnormalities caused by mental diseases (Yao et al., 2017), which can be assessed by the dynamic FC (dFC) approach. The dFC method can precisely describe the collaboration of brain regions by measuring the time-varying covariance of their neural signals during resting-state (Yao et al., 2017). The dFC method has been used to measure the dynamic connectivity abnormalities in several psychiatric and neurological disorders, such as depression (Kaiser et al., 2016; Pang et al., 2018), autism (Guo et al., 2018), schizophrenia (Supekar et al., 2019), and epilepsy (Li et al., 2018c), and provide evidence that altered fluctuating communication among high-order regions or networks is associated with the pathological symptoms of these respective disorders. Only a few studies have investigated the dynamic changes of brain connectivity in patients with GAD using the dFC approach. These studies revealed that the altered temporal features of dFC can be used as effective features to distinguish adolescents with GAD from healthy controls (HCs) with high accuracy (Yao et al., 2017). In addition, dFC can be used to identify the differences in brain states and network properties between GAD and comorbid GAD patients with insomnia (Li et al., 2018a). Both dFC-related studies of GAD have focused on time-varying brain connectivity changes between networks derived from independent component analysis, and they highlight the importance of considering fluctuating dynamic neural communication among brain systems when studying the pathophysiological mechanism of GAD. Recently, dynamic FC density (dFCD) has also been used to characterize the abnormal dynamic neural communication among brain regions in individuals with psychiatric and neurological disorders, such as in children with benign epilepsy with centrotemporal spikes (Li et al., 2018d). dFCD was based on static FCD that is defined by the functional connections of each voxel with all other voxels in the whole-brain (Tomasi and Volkow, 2010) and sliding window correlation approach. Unlike the seed-based FC approach, FCD is an unbiased graph theory method (Zhang et al., 2017) that does not require any prior assumption (Pang et al., 2017) and is suitable for exploratory analyses (Tomasi et al., 2016). In specific, a prior study found that patients with GAD exhibit aberrant frequency-specific FCD (Zhang et al., 2017). In addition, dFCD can be used to depict voxel-wise FC changes within shorter time scales than static FCD. Therefore, dFCD may be a prominent approach to provide more subtle and complementary information compared with previous FC and static FCD-related findings to deepen our understanding of patients with GAD.

In this study, we employed the unbiased whole-brain global dFCD approach to characterize the abnormal communication among brain regions in patients with GAD. The standard deviation (SD) of FCD values across sliding windows was utilized to quantify the alterations of dFCD. According to aforementioned studies, patients with GAD exhibit abnormal static FCD and dynamic characteristic of brain connectivity

during resting state. We expect that patients with GAD will also show abnormal patterns of time-varying FCD.

## 2. Materials and methods

### 2.1. Participants

In total, 90 patients with GAD and 88 HCs participated in this study. The patients were enrolled from the Clinical Hospital of Chengdu Brain Science Institute, University of Electronic Science and Technology of China (UESTC). Two experienced psychiatrists interviewed the patients using the Structured Clinical Interview for DSM-IV-TR-Patient Edition (SCID-P, 2/2001 revision). All patients included in this study met the DSM-IV criteria for GAD. Exclusion criteria included schizophrenia, major depressive disorder, personality disorder, substance abuse, neurological illness and any history of head trauma or mental retardation. In specific, considering that the pathological mechanisms of patients with GAD and those comorbid GAD with major depressive disorder may be different, we excluded those patients with comorbidity of anxiety and depression to reduce the heterogeneity of the patients. The clinical states of each patient were evaluated using the 14-item Hamilton Anxiety Rating Scale (HAMA). Most patients received medication treatment, including selective serotonin reuptake inhibitors (SSRIs), serotonin and norepinephrine reuptake inhibitors (SNRIs), and norepinephrine and selective serotonin reuptake inhibitors (NaSSRI). The detailed medical information of the patients is presented in Table 1. No patients were undergoing psychotherapy at the time of the study. The HCs were enrolled from the local community by using advertisements and screened with the SCID non-patient edition. The HCs had no history of any psychiatric illness or neurological disorders. The between-group differences in gender, age, years of education, and head motion were

**Table 1**  
Characteristics of demographic and clinical variables.

Variables	HC (n = 80)	GAD (n = 81)	Statistics	p-value
Age (years)	37.95 ± 14.49	38.28 ± 11.50	$U = 3102$	0.64 <sup>a</sup>
Gender (male / female)	38/42	33/48	$\chi^2 = 0.75$	0.39 <sup>b</sup>
Handedness (left / right)	3/77	2/79	$\chi^2 = 0.22$	0.64 <sup>b</sup>
Education (years)	13.08 ± 3.37	12.85 ± 3.06	$U = 2770$	0.10 <sup>a</sup>
Mean FD	0.09 ± 0.04	0.09 ± 0.05	$U = 2863$	0.20 <sup>a</sup>
Duration of illness (months)	–	45.59 ± 57.50	–	–
Age of first onset (years)	–	34.58 ± 11.61	–	–
No. of anxiety episodes	–	1.95 ± 0.98	–	–
Duration of single anxiety episode	–	5.70 ± 5.69	–	–
HAMA score	–	24.02 ± 5.85	–	–
Medical				
Medication load index		1.64 ± 0.73		
Medications, no. of patients				
SSRIs				
Fluoxetine		1		
Sertraline		10		
Paroxetine		25		
citalopram		1		
Escitalopram		12		
Fluvoxamine		1		
SNRIs				
Venlafaxine		5		
Duloxetine		15		
NaSSRI				
Mirtazapine		2		

Abbreviations: HC, healthy control; GAD, generalized anxiety disorder; FD, framewise displacement; HAMA, 14-item Hamilton anxiety rating scale; SSRIs, selective serotonin reuptake inhibitors; SNRIs, serotonin and norepinephrine reuptake inhibitors; NaSSRI: norepinephrine and selective serotonin reuptake inhibitors.

Values are mean ± standard deviation.

<sup>a</sup> Mann–Whitney  $U$  test.

<sup>b</sup> Chi-square  $t$ -test.

not significant. All participants were informed about the procedures and details of the study and provided written informed consent. This study was approved by the research ethical committee of the UESTC, listed on Clinical-Trials.gov (Registration Number: NCT02888509).

Nine patients with GAD and eight HCs were excluded because of maximal head motion or displacement exceeding 2.5 mm or to head rotation exceeding 2.5° during fMRI scanning. Finally, 81 patients with GAD and 80 HCs were included in this study. The demographic and clinical characteristics of the two groups and their differences in gender, age, handedness, years of education, and mean framewise displacement (FD) are presented in Table 1.

## 2.2. Data acquisition

MRI data were obtained using a 3T GE DISCOVERY MR750 scanner (General Electric, Fairfield Connecticut, USA) with an eight-channel prototype quadrature birdcage head coil. Participants were instructed to rest with their eyes closed, not to fall asleep, not to think of anything, and to keep their head motionless during scanning. We asked all participants if they had fallen asleep or opened their eyes during scanning, and we excluded those who answered yes. Finally, no patients were excluded because of opening their eyes or falling asleep during scanning. Resting-state functional images were collected using an echo-planar imaging sequence with the following parameters: repetition time (TR)/echo time = 2000/30 ms, matrix size =  $64 \times 64$ , flip angle =  $90^\circ$ , field of view =  $240 \text{ mm} \times 240 \text{ mm}$ , voxel size =  $3.75 \text{ mm} \times 3.75 \text{ mm} \times 3.2 \text{ mm}$ , slices = 43, slice thickness = 3.2 mm, no gap, and a total of 255 volumes.

## 2.3. fMRI data preprocessing

The Data Processing and Analysis of Brain Imaging (DPABI v3.0) (<http://rfmri.org/dpabi>) toolbox was used to preprocess the functional imaging data. For each subject, the first five volumes were discarded to ensure the equilibrium of the signal. Subsequently, the slice timing correction and head motion realigning were performed on the remaining 250 volumes. The remaining images were further spatially normalized to a standard template for Montreal Neurological Institute and resampled to a  $3 \text{ mm} \times 3 \text{ mm} \times 3 \text{ mm}$  resolution. The normalized images were then linearly detrended to reduce the effects of signal drifts. Furthermore, nuisance covariates (Friston-24 parameters of head motion, white matter signal, cerebrospinal fluid signal and global signal) were regressed out from the data. Previous studies reported that the global signal regression can enhance the specificity of FC calculation (Chen et al., 2016; Fox et al., 2009), in eliminating the non-neuronal signals of global variance related to physiological noise (Birn, 2012) and motion artefact (Murphy and Fox, 2017; Power et al., 2014; Yan et al., 2013). Therefore, we regressed the global signal in our study. In particular, to avoid inducing artificial local spatial correlation, we did not smooth on our data. Subsequently, data were subjected to band pass filtering at a frequency range of 0.01–0.08 Hz. Finally, motion scrubbing with cubic spline interpolation was performed on the data.

## 2.4. Head motion analysis

The mean FDs of the GAD group ( $0.09 \pm 0.05$ ) and HC group ( $0.09 \pm 0.04$ ) were calculated on the basis of realignment parameters to assess the confounding influence of head motion on connectivity measures. The between-group difference in mean FD was not significant ( $p = 0.20$ ) as determined using a two-sample *t*-test. In addition, scrubbing analysis was employed to detect the “bad” time points with  $\text{FD} > 0.5 \text{ mm}$  (Power et al., 2013). The bad time points and their 1-back and 2-forward time points were discarded (number of GAD:  $5.63 \pm 12.99$ ; number of HCs:  $4.90 \pm 8.78$ ) from the time series of each subject, and the data for these missing points were estimated

through cubic spline interpolation (Pang et al., 2018; Wise et al., 2017). The between-group difference in remaining time points after discarding those “bad” points was not significant (Mann–Whitney *U* test,  $p = 0.90$ ).

## 2.5. Dynamic functional connectivity density analysis

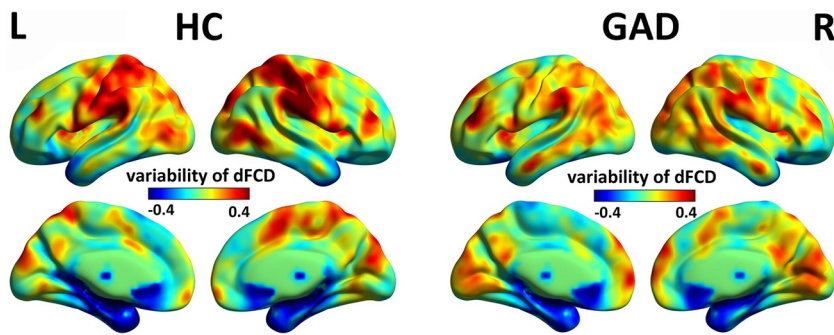
The sliding window dFCD approach was applied to obtain the dynamic functional maps for each participant via DynamicBC (Liao et al., 2014). The window length is a key parameter in sliding window correlation calculation. To avoid introducing spurious fluctuations in dFCD, the minimum window length should no less than  $1/f_{\min}$ , where  $f_{\min}$  is the minimum frequency of time series (Leonardi and Van De Ville, 2015). At the same time, the window length should not be too long to disrupt the temporal variability dynamic of FCD (Li et al., 2018b). Basing from previous studies, we selected 50 TRs as the window length to optimize the balance between capturing a rapidly shifting dynamic relationship and achieving reliable estimates of the correlations between regions (Li et al., 2018b,c; Pang et al., 2018). The entire resting-state series of 250 TRs was divided into 41 windows using 50 TRs (100 s) as the window length and 5 TRs (10 s) as the step size. We obtained a global FCD map in each window by computing Pearson's correlations between the truncated time course of all pairs of voxels within the automated anatomical labelling-90 (AAL-90) atlas (comprising 45 cortical and subcortical regions in each hemisphere) (Tzourio-Mazoyer et al., 2002), yielding a set of sliding-window FCD maps for each subject. We used  $r = 0.2$  as the correlation coefficient threshold to define the connectivity between two voxels. If their correlation coefficient was larger than 0.2, then connectivity was present between them. The threshold was selected to eliminate the weak correlations induced by noise (Li et al., 2018b). Subsequently, the temporal variability was estimated by computing the SD of FCD across sliding windows. In consideration that the global signal regression may induce controversial negative correlations (Fox et al., 2009; Murphy et al., 2009), all our analyses were performed based on positive correlations above a threshold of 0.2.

## 2.6. Statistical analysis

Before starting the statistical analyses, the temporal variability map of each subject was normalized into a z-score matrix by subtracting the mean and dividing it by the SD of the global values within the AAL-90 atlas. Then, the normalized images were smoothed using a  $6 \text{ mm} \times 6 \text{ mm} \times 6 \text{ mm}$  full-width at half maximum Gaussian kernel. Subsequently, the two-sample *t*-test was performed to detect the between-group difference in dFCD variability patterns. Gender, age, years of education, and mean FD were included as covariates. Statistical maps of the between-group difference were thresholded using permutation tests as implemented in Permutation Analysis of Linear Models (Winkler et al., 2016) and integrated into the DPABI toolbox. Multiple comparison was then performed on the basis of threshold-free cluster enhancement (TFCE) with 5000 permutations (two-tailed,  $p < 0.05$ ). A permutation test with TFCE was considered as a prominent method to achieve the best balance between the family-wise error rate and the test-retest reliability (Chen et al., 2018). Regions surviving the multiple comparison correction were selected as regions of interest (ROIs) and subjected to the following analyses.

## 2.7. Support vector regression prediction for symptom severity of patients

A support vector regression (SVR) model was trained to estimate the symptom severity for each patient based on the temporal variability of dFCD. The symptom severity of patients was measured by their HAMA score. In particular, we applied the epsilon-SVR, implemented in LIBSVM toolbox (Chang and Lin, 2011). Subsequently, the altered voxel-wise temporal variability of dFCD for each ROI in the GAD group



**Fig. 1.** Spatial distribution maps of dFCD variability in the HC and GAD groups. The dFCD variability are normalized by subtracting the mean and dividing it by the SD of the global values and averaged across participants within each group. dFCD, dynamic functional connectivity density; HC, healthy control; GAD, generalized anxiety disorder; SD, standard deviation; L, left; R, right.

was selected as features during prediction. Then, we employed a leave-one-out cross validation (LOOCV) to train a model that could be used to estimate each patient's HAMA score. Suppose that  $N$  samples are present in each LOOCV, we selected the data of  $N-1$  patients as the training set to train the model and the remaining data as the test set to obtain the HAMA score using the model. For each ROI, the LOOCV calculation procedure was repeated  $N$  times so that all patients' HAMA scores could be predicted. Subsequently, the Pearson's correlation between real and estimated HAMA scores was performed to obtain the correlation coefficient value  $R$ . Finally, we performed a nonparametric permutation test to assess the statistical significance of the result. In each trial of the permutation, the actual HAMA scores were randomly reshuffled among the patients, and the abovementioned procedure for SVR prediction was repeated to obtain a new correlation coefficient value  $R_{perm}$ . We repeated this procedure 5000 times and counted the number of  $R_{perm}$  that were larger than the original  $R$ , and the ratio between this number and 5000 was used to determine the final  $p$  value.

## 2.8. Clinical variable-related correlation analysis

The medication information of each patient was assessed using a total medication load index, of which the calculation of the index was described in previous studies (Han et al., 2019; Pang et al., 2018; Redlich et al., 2015; Wang et al., 2018c). Each medication can be divided into level 1, 2, 3, or 4 in accordance with a previously employed method (Sackeim, 2001), with reference to the daily dose range and duration of the medication. Detailed information on the conversion between medication dosage and corresponding levels is shown in the tables in the Appendix of Sackeim et al. Medications on levels 1 and 2 were coded as low dose, whereas those with levels 3 and 4 as high dose. Patients not taking these medications were added to a no-dose subtype. Then, each medication was coded as 0 (absent), 1 (low dose), or 2 (high dose) according to the level of the medication. Two medications not mentioned by Sackeim, escitalopram and duloxetine were coded as 0, 1, or 2 according to the midpoint of the daily dose range recommended by the Physician's-Desk-Reference. Finally, the sum of the codes of all medications one patient had taken was used as the total medication load index of that patient. This index could then be used to reflect the dose of medications patients have taken. To estimate the possible effects of the medications on the dFCD variability-related results, we performed the non-parametric Spearman's rank correlation between the total medication load index and the dFCD variability of each ROI with significant between-group differences. The threshold of  $p < 0.05/5$  (Bonferroni correction) was employed as the statistically significant level for the correlation analyses.

## 2.9. Validation analyses

We verified our findings of dFCD variability with 50 TRs (100 s) as the window length and 5 TRs (10 s) as the step size in sliding-window correlation analyses. We changed the window length and calculated the dFCD variability with 30 TRs (60 s) and 80 TRs (160 s) as the window

length and 5 TRs (10 s) as the step size. Meanwhile, to test the confounding influence of different step size on our main findings, we computed the dFCD variability with 1 TR (2 s) and 3 TRs (6 s) as the step size and 50 TRs (100 s) as the window length. The corresponding results are shown in the supplementary materials.

## 3. Results

### 3.1. Demographics and clinical characteristics

The demographic and clinical information of the HCs and patients with GAD are presented in Table 1. The differences in gender ( $\chi^2$  test,  $p = 0.39$ ), handedness ( $\chi^2$  test,  $p = 0.64$ ), age (Mann-Whitney  $U$  test,  $p = 0.64$ ), years of education (Mann-Whitney  $U$  test,  $p = 0.10$ ), and mean FD (Mann-Whitney  $U$  test,  $p = 0.20$ ) were not insignificant (Table 1).

### 3.2. Spatial distribution maps of dFCD variability of the HC and GAD groups

The spatial distribution maps of dFCD variability of HC and GAD groups were similar (Fig. 1). Regions with high dFCD variability were mainly located in the prefrontal, parietal, somatosensory, and visual cortices, and regions with low dFCD variability were mainly involved in the temporal gyrus, the subgenual anterior cingulate cortex, the hippocampus, and the thalamus.

### 3.3. Whole gray matter dFCD variability changes in GAD

Group-level statistical analysis revealed that patients with GAD exhibited increased temporal dFCD variability in the bilateral dorsomedial prefrontal cortex (dmPFC) and left hippocampus while decreased temporal dFCD variability in the right postcentral gyrus (PoG) ( $p < 0.05$ , TFCE corrected) (Fig. 2, Table 2).

### 3.4. Abnormal dFCD variability predicts symptom severity of GAD

We investigated the relationship between the altered temporal variability of dFCD and symptom severity of patients with GAD using SVR and LOOCV. The statistical significance of the result was assessed using permutation test ( $p < 0.05/5$  was statistically significant, Bonferroni correction). The abnormal dFCD variability in the left dmPFC could predict the symptom severity of GAD (Bonferroni corrected  $p = 0.0098$ ) (Fig. 3). However, other regions with significant group differences in dFCD variability did not show any significant correlation.

### 3.5. Effects of medications on dFCD variability-related analysis

We did not find any significant correlations between the total medication load index of GAD and the abnormal dFCD variability in each ROI.

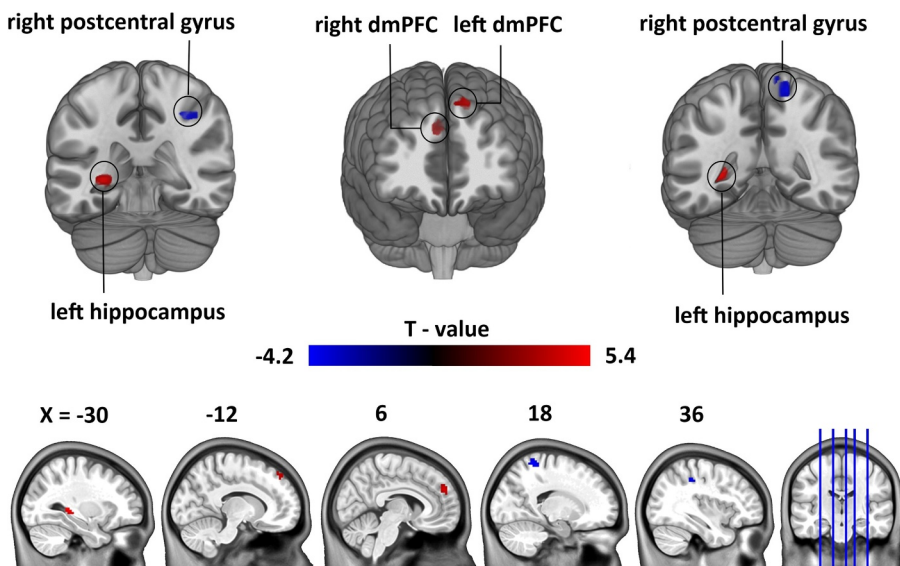


Fig. 2. Group differences in dFCD variability between GAD patients and HCs ( $p < 0.05$ , TFCE corrected). Significant differences were anchored in the bilateral dmPFC, left hippocampus and right postcentral gyrus. dFCD, dynamic functional connectivity density; GAD, generalized anxiety disorder; HCs, healthy controls; dmPFC, dorsomedial prefrontal cortex.

**Table 2**  
Group differences in dFCD variability between GAD patients and HCs.

Brain regions	sphere L/R	Cluster size (voxels)	Peak t values	Coordinates in MNI		
				x	y	z
Hippocampus	L	32	5.40	-30	-39	3
dmPFC	L	18	4.08	-12	45	48
dmPFC	R	37	4.66	6	51	30
Postcentral gyrus	R	36	-4.22	18	-42	63
Postcentral gyrus	R	45	-3.94	36	-33	45

Abbreviations: dFCD, dynamic functional connectivity density; GAD, generalized anxiety disorder; HCs, healthy controls; L, left; R, right; dmPFC, dorsomedial prefrontal cortex.

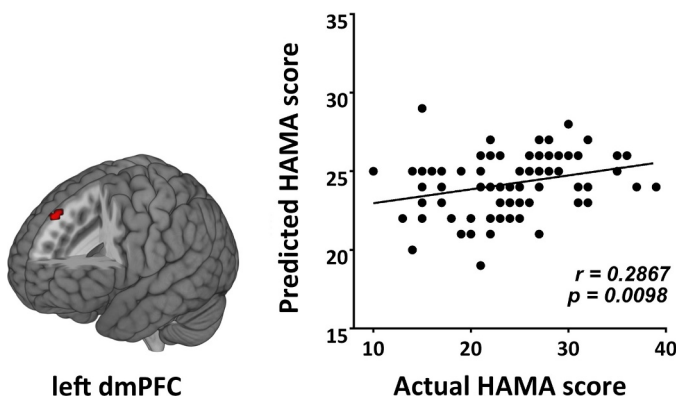


Fig. 3. The abnormal dFCD variability of the left dmPFC could predict the symptom severity of GAD (Bonferroni corrected  $p = 0.0098$ ). The correlation between the actual HAMA score and the predicted HAMA score of GAD was calculated using Pearson's correlation analysis. dFCD, dynamic functional connectivity density; dmPFC, dorsomedial prefrontal cortex; GAD, generalized anxiety disorder; HAMA, 14-items Hamilton Anxiety Rating Scale.

3.6. Validation analyses

In the validation analyses, the group differences of dFCD variability with different sliding window lengths and step sizes remained similar to the main findings obtained using a sliding window length of 50 TRs and step size of 5 TRs (supplementary Tables S1–S4, and Figures S5–S8). Moreover, the abnormal dFCD variability of the left dmPFC could predict the symptom severity of GAD even when the step size of sliding widow was changed (supplementary Figure S9 and Figure S10), but the

predictive effect was not significant when the sliding window length was changed. In addition, the effects of medications on dFCD variability-related group differences were not significant during validation analyses.

4. Discussion

In this study, we investigated the abnormal patterns of dynamic FCD in patients with GAD for the first time. Alterations of brain connectivity in patients with GAD were predominantly derived from previous static FC-related studies that ignored the dynamic properties of FC. Only a few network-based dynamic FC-related studies have so far focused on time-varying changes of FC in patients with GAD, and the whole-brain voxel-wise dynamic connectivity alterations in patients with GAD have not yet been addressed. Our results complemented previous static FC-related findings and extended dynamic FC-related findings from the whole-brain perspective using the dFCD approach, which did not require any prior hypothesis. We found that patients with GAD exhibited increased dFCD variability in the medial prefrontal cortex and hippocampus, which are associated with self-referential processing and emotional memory. Meanwhile, the dFCD variability of GAD decreased in the PoG, a brain region implicated in sensorimotor function. These findings provide novel evidence to deepen our understanding of patients with GAD by detecting abnormal dynamic patterns of brain communications among regions without requiring any assumption.

Patients with GAD showed increased dFCD variability in the dmPFC, one of the key regions of the DMN (Northoff et al., 2006; Raichle, 2015). The DMN is responsible for self-related cognition, emotional processes, and future planning (Menon, 2011; Sylvester et al., 2012), and it is more active at rest than performing cognitive tasks (Fox et al., 2005; Raichle, 2015; Raichle et al., 2001). The DMN has received increasing attention in numerous psychiatric diseases, and the abnormal resting state FC (rsFC) within DMN as well as between DMN and other core neurocognitive brain networks has been reported in patients with schizophrenia (Krishnadas et al., 2014; Supekar et al., 2019), depression (Dong et al., 2019; Mulders et al., 2015), and obsessive-compulsive disorder (Fan et al., 2017). Patients with GAD also exhibit functional and structural impairments of the DMN, including altered rsFC (Sylvester et al., 2012; Wang et al., 2016) and aberrant gray matter volume (Schienle et al., 2011). The DMN is also closely correlated with self-referential mental activity (Raichle, 2015). Anxiety disorders are considered as “distress disorders” that are associated with negative self-referential processes, including worry, rumination, and self criticism (Renna et al., 2017). Several

anxiety disorders are closely correlated with aberrant self-referential processing, involving social anxiety disorder (Brown et al., 2019; Cui et al., 2017; Yoon et al., 2019), social phobia (Blair and Blair, 2012; Blair et al., 2011), and GAD (Fresco et al., 2017; Mennin et al., 2018). The abnormal self-referential processing of these anxiety disorders is mainly associated with dysfunction of the medial prefrontal cortex. The abnormal dFC in the dmPFC in GAD found here may reflect the disrupted dynamic connectivity patterns of the DMN, which may be associated with the negative self-referential processing of GAD. Basing on this abnormal pattern, we could predict the symptom severity of GAD, indicating that the altered dynamic neural communication between the dmPFC and other brain regions may be associated with the symptom changes of the disease. dFC variability increased in the left hippocampus of patients with GAD. The hippocampus is a critical region of the limbic system that is involved in emotion processing and important for consolidation and retrieval of emotional memory (Reshetnikov et al., 2018). Patients with GAD often experience emotional hyperarousal and are especially sensitive to threatening-related emotional stimuli (Mochcoyitch et al., 2014). Several brain regions, including the orbitofrontal cortex (Pujara et al., 2019), the cingulate cortex, the insula, the amygdala, and the hippocampus, are implicated in threat processing (Fiddick, 2011), and patients with GAD exhibit hyperactivation in the hippocampus when presented with pictures correlated to life-threatening behaviors (Moon and Jeong, 2015). The decreased rsFC between the hippocampus and regions involved in limbic-prefrontal circuitry has also been reported in patients with GAD (Chen and Etkin, 2013). Several studies found that the volume of the hippocampus is decreased in patients with GAD (Abdallah et al., 2013; Hettema et al., 2012; Moon et al., 2014). The excessive dFC variability of the hippocampus in patients with GAD found in the present study may reflect unstable dynamic functional integration of the limbic system that is formed by the hippocampus and other regions, which may also be associated with extensive sensitivity to threatening stimuli of GAD.

Furthermore, the dFC variability of GAD decreased in the right PoG, a brain region involved in the sensorimotor network (Jiang et al., 2019; Wang et al., 2018b; Zhu et al., 2019). Previous neuroimaging studies showed that the rsFC (Cui et al., 2016), spontaneous regional brain activity (Xia et al., 2017), and brain signal variability (Li et al., 2019) of the sensorimotor areas are decreased in patients with GAD, suggesting the functional impairments of this network. The reduced dFC variability of the sensorimotor area in patients with GAD may signify weakness in neural communication between this network and other regions of the brain, which is consistent to the functional abnormalities of this region.

Several limitations of this study should be considered. First, albeit the dFC method has been broadly used in numerous psychiatric and neurological disorder-related studies, the neurocognitive functioning represented by this approach is still ambiguous (Li et al., 2018b). Second, the window size applied in this study was adopted from previous studies (Li et al., 2018b, 2018c; Pang et al., 2018). Although the between-group differences in dFC variability with different sliding window lengths and step sizes were less influenced by these factors, the selection of these parameters remains controversial. The significant prediction of the symptom severity of GAD only survived in the sliding window length of 50 TRs, which may be affected by several factors, including the selection of the predictive model and the sliding-window parameters, which need to be further verified in future studies. Third, the important regions of GAD, including the ventromedial prefrontal cortex (vmPFC) and amygdala, which play important roles in emotional dysregulation and pathological worry in GAD, did not exhibit abnormal dFC variability in our study. We assumed that the dFC method is insensitive to the abnormalities of the vmPFC and amygdala of GAD. Their abnormalities may be stable and not fluctuate within a short period of time. The other explanation is that our sample size hinders our observation of relevant results. Fourth, given the high comorbidity of

GAD and major depressive disorder, excluding those comorbid patients may lead to uncomprehensive findings. Comorbidity samples are required to further test our findings in the future. Collecting eyes open and eyes closed information via self-report may have some limitations. For example, participants who reported that they stayed awake with eyes closed may actually fall asleep for a short while without awareness. In addition, some patients who fell asleep during scanning may cheat us that they were awake. These issues cannot be checked by self-report. Therefore, falling sleep and/or opening eyes are still possible confounders having effects on our results. Future studies are expected to further investigate the influences of these conditions on our results. And most of our patients have been treated with medications. Future studies are expected to rule out the potential confounding effects from recruiting large samples of medication-free patients and verify our findings. In addition, the time of stable medication treatment for every patient prior to the scanning may have a potential effect on our results. Future studies are expected to assess the influence of this indicator on the FC in GAD.

## 5. Conclusion

Patients with GAD exhibited altered brain functional dynamics using the voxel-wise FCD and sliding window correlation approach. The altered dynamics of functional connectivity was located in the dmPFC, hippocampus, and sensorimotor area. The dFC abnormalities of the dmPFC may be potential neuromarker in predicting the anxiety symptom severity of GAD. These findings suggest the importance of investigating the time-varying fluctuations of brain functional communication to improve our understanding of GAD.

## CRedit authorship contribution statement

**Chen Yuyan:** Conceptualization, Data curation, Methodology, Formal analysis, Writing - original draft, Writing - review & editing. **Cui Qian:** Conceptualization, Methodology, Writing - review & editing, Funding acquisition. **Xie Ailing:** Data curation, Methodology, Formal analysis. **Pang Yajing:** Supervision, Writing - review & editing. **Sheng Wei:** Data curation, Software, Methodology, Formal analysis. **Tang Qin:** Data curation, Methodology, Validation. **Li Di:** Methodology, Validation. **Huang Jing:** Methodology, Validation. **He Zongling:** Data curation, Writing - review & editing, Funding acquisition. **Wang Yifeng:** Supervision, Funding acquisition. **Chen Huafu:** Conceptualization, Methodology, Writing - review & editing, Funding acquisition.

## Declaration of Competing Interest

All authors declare that they have no conflicts of interest.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:[10.1016/j.jad.2019.09.084](https://doi.org/10.1016/j.jad.2019.09.084).

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