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Neural mechanisms of aberrant self-referential processing in patients with generalized anxiety disorder

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ABSTRACT

Massive theoretical studies in clinical psychology have implicated the self in understanding internalizing disorders (i.e., anxiety and mood disorders), in which self-related tasks were frequently used to investigate internalizing psychopathology. As one of the most frequently seen internalizing disorder in primary care, patients with generalized anxiety disorder (GAD) are characterized by inappropriate self-related processing such as negative self-referential thinking. However, relevant neural mechanisms remain unknown. In this study, participants underwent a self-related task which they were presented with several positive and negative trait words and were required to judge the extent to which these traits matched themselves when compared to their average peers. Aberrant brain activation and functional connectivity of GAD were detected during processing positive and negative traits. Compared to healthy controls (HCs), patients with GAD exhibited abnormal self-processing which manifested as lower biased self-rating scores particularly for negative traits and weaker brain activity in the left dorsomedial prefrontal cortex, inferior frontal gyrus, superior temporal sulcus (STS), and bilateral lingual gyrus when processing trait words. Abnormal functional connections between these hypoactive regions and regions associated with reward, emotion, and theory of mind were observed in subsequent psychophysiological interaction analysis. An attenuation of connectivity between the left insula and left STS was associated with greater severity of anxiety symptom in GAD patients. These findings provide insight into the abnormal neurocognitive mechanisms of biased self-related processing in GAD patients, which involves distorted self-schema accompanied by abnormal activation and functional connections of regions implicated in self-related and social cognition processing.

1. Introduction

Generalized anxiety disorder (GAD) is a common and debilitating psychological disorder, in which patients experience excessive and uncontrollable worry, alongside physical and psychological symptoms such as restlessness, muscle tension, and insomnia, fatigue (DeMartini et al., 2019). GAD is characterized by persistent negative self-referential thinking (NSPR), involving worry, rumination, and self-criticism (Fresco et al., 2017; Mennin et al., 2018) and high trait negative affectivity (Brown et al., 1998; Clark et al., 1994). These distorted cognitive patterns play important roles in the pathological development of GAD (Mennin et al., 2018).

GAD is underscored by impairments in emotional regulation and cognitive control. (Hallion et al., 2017; Mochcoyitch et al., 2014). Aberrant self-related processing is involved in the development and maintenance of internalizing symptoms of anxiety disorders (Lin et al., 2018). Specifically, negative self-related processing underpins the course and severity of GAD (Mennin et al., 2018), and can detrimentally affect treatment efficacy (Mennin and Fresco, 2013; Mennin et al., 2018). To date, clinical findings on changes in self-related processing in anxiety disorders have predominantly focused on patients with social anxiety disorder (SAD) (Blair et al., 2008, Blair et al., 2011, Boehme

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et al., 2015). A summary of functional magnetic resonance imaging (fMRI)-related findings of SAD by Lin and colleagues (Lin et al., 2018) revealed that patients with SAD tend to use negative self-related evaluation when exposed to social stimuli and exhibit abnormal brain activity in regions implicated in cortical midline structures, default mode network, and limbic system.

The self is a complex concept, which facilitates awareness of existence (Weiler et al., 2016). Among numerous concepts of self, self-referential processing (SRP) is presumed to be core feature (Northoff et al., 2006), a defining characteristic of human beings (Lackner and Fresco, 2016). The SRP could reflect the experience strongly associated with one's own person and serve as the basic function in discriminating self and others (Lin et al., 2018). Previous findings suggest that SRP was positively correlated with social cognitive capacities such as theory of mind (TOM) and empathic accuracy (Dinulescu et al., 2021), in which TOM reflect one's ability to infer mental states of others. Healthy SRP is essential for adaptive social cognition and self-regulation (Mennin and Fresco, 2013). Healthy individuals with optimistic cognitive biases often exhibited a stronger self-referential effect for positive than negative words (Durbin et al., 2017). Whereas, negative associations of SRP with depressive symptoms have been found in college students (Dinulescu et al., 2021). Neuroimaging findings demonstrated that fronto-striato circuit plays a pivotal role in relevant processing in self-related task (Yamada et al., 2013). Significant neural activation of the medial prefrontal cortex (MPFC) and anterior cingulate cortex (ACC) was observed in healthy subjects when judging the extent to which given traits matched themselves (Moran et al., 2006). Healthy individuals showing strong self-positive bias were observed to exhibit greater activation in the MPFC (Fields et al., 2019) but weaker brain activation in the orbitofrontal cortex and dorsal ACC (Beer and Hughes, 2010).

Conversely, inordinate NSRP was evidenced to have destructive effects on mental health, served as a transdiagnostic factor for multiple affective psychopathologies (i.e., anxiety and depression) (Kircanski et al., 2015). Rumination, worry, and self-criticism are most commonly forms of NSRP (Dinulescu et al., 2021), receiving a number of theoretical and empirical attention in psychiatric illness. Notably, patients with GAD were featured by intense emotional experiences coupled with excessive NSRP, but fail to down-regulate their emotions and somatic responses (Fresco et al., 2017). Previous studies have revealed associations between greater NSRP and higher GAD symptoms in college students (Lackner and Fresco, 2016; Tracy et al., 2021), and reported large contribution of NSRP in suicidal thoughts and behaviors in adolescent cohort (Allison et al., 2021). Despite the critical role of NSRP in understanding anxiety and mood disorders, NSRP cannot be identified in the Diagnostic and Statistical Manual of Mental disorders (DSM-5) (Mennin and Fresco, 2013). At present, only a few sporadic behavioral findings point out the important role of NSRP in the pathological development of GAD, the underlying neural substrates remain obscure.

This study employed a self-related task (superiority illusion task) adopted by Yamada (Yamada et al., 2013) to investigate the neural basis underlying self-related abnormalities in GAD. We hypothesized that patients with GAD would exhibit abnormal brain activation and functional organization during self-related processing, and that these abnormalities would be correlated with clinical performance of GAD.

2. Methods

2.1. Participants

In total, 35 patients with GAD and 36 healthy controls (HCs) were recruited. All participants had normal or corrected-to-normal vision. Patients were recruited from the Clinical Hospital of Chengdu Brain Science Institute, University of Electronic Science and Technology of China (UESTC). Each patient was interviewed by two experienced psychiatrists using the Structured Clinical Interview for DSM-IV-TR-Patient Edition (SCID-P, 2/2001 revision) and met the criteria for GAD. Patients with schizophrenia, personality disorder, substance abuse, neurological illness, or history of loss of consciousness were excluded. Severity of anxiety of patients with GAD was measured using the 14-item Hamilton Anxiety Rating Scale (HAMA). Most patients were treated with medication (detail information is presented in Table 1). All HCs were recruited from the local community via advertisements. HCs were free of any history of psychopathologic conditions, as assessed by the SCID non-patient edition. GAD and HC groups were matched for age, sex, handedness, and mean framewise displacement during the task. Detailed demographic information regarding GAD patients and HCs is presented in Table 1. This study was approved by the ethical committee of the UESTC and is listed on Clinical-Trials.gov (Registration Number: NCT02888509). All participants were provided a complete description of the study and detailed explanations with examples to ensure they fully understood the instructions prior to scanning. All participants provided written informed consent prior to participation.

Table 1
Characteristics of demographic and clinical variables of HCs and patients with GAD.

Variables	HC (n = 36)	GAD (n = 35)	Statistics	p-value
Age (years)	33.08 ± 11.03	36.40 ± 9.27	U = 512	0.18[a]
sex (male / female)	18/18	13/22	$\chi^2 = 1.19$	0.27[b]
Handedness (left / right)	0/36	1/34	$\chi^2 = 1.04$	0.31[b]
Education (years)	13.61 ± 3.13	12.40 ± 3.18	U = 429	0.02[a]
mean FD of run1	0.09 ± 0.04	0.10 ± 0.06	U = 620	0.91[a]
mean FD of run2	0.10 ± 0.05	0.10 ± 0.06	U = 626	0.98[a]
mean FD of run3	0.09 ± 0.04	0.11 ± 0.07	U = 560	0.42[a]
Mean FD of 3 runs	0.09 ± 0.04	0.10 ± 0.06	U = 615	0.86[a]
Duration of illness (months)	–	48.83 ± 68.93	–	–
Age of first onset (years)	–	32.46 ± 9.58	–	–
No. of anxiety episodes	–	2.26 ± 1.46	–	–
Duration of single anxiety episode	–	3.91 ± 2.95	–	–
HAMA score	–	24.31 ± 5.06	–	–
Positive SI scores	1.27 ± 0.86	0.96 ± 1.00	$t_{(69)}$ = 1.42	0.16[c]
Negative SI scores	0.94 ± 0.77	0.29 ± 1.02	$t_{(69)}$ = 3.01	<0.01[c]
Mean SI scores	1.11 ± 0.70	0.63 ± 0.83	U = 394	0.01[a]
Medical				
Total medication load index		1.57 ± 0.74		
Antianxiety medications, no. of patients				
Fluoxetine		1		
Sertraline		1		
Paroxetine		13		
citalopram		1		
Escitalopram		8		
Fluvoxamine		1		
Venlafaxine		3		
Duloxetine		2		

HC, healthy controls; GAD, generalized anxiety disorder; FD, framewise displacement; HAMA, 14-item Hamilton anxiety rating scale; SI, superiority illusion; $t_{(df)}$, between-group t statistic and degrees of freedom.
Values are mean ± standard deviation.
[a] Mann–Whitney U test.
[b] Chi-square test.
[c] Two-sample t-test.

2.2. Task paradigms

Participants completed a self-related task (Yamada et al., 2013) in an event-related design during fMRI data acquisition. The task was programmed using *E*-prime software. In this task, 60 personality words were presented to each participant, in which half of the personality words (traits) were of positive valence and the other half were of negative valence. These traits were derived from a prior study (Rosenberg et al., 1968) and were translated into Chinese. Participants were instructed to rate each trait by comparing with their average peers. For each trait, participants viewed the screen with the numbers −5, −4, −3, −2, −1, 0, 1, 2, 3, 4, 5 (11-point scale), and provide a rating for their choice using a button through their hand (−5 = extremely lower than average; 0, equal to average; 5 = extremely higher than average). The rating score could help reflecting the superiority illusion of participants on given traits. The self-rating score (superiority illusion score) for a positive trait was obtained by subtracting 5 from the rating score for this trait (score-5). The self-rating score for a negative trait was obtained by subtracting the rating score for this trait from 5 (5-score). The scores of participants were used as an indicator of the extent of their superiority illusion, whereby higher scores implied that individuals evaluated themselves as superior to average peers in traits (possessing stronger positive traits and weaker negative traits).

The self-related task comprised three rounds. The order of rounds was random and counterbalanced across participants. Each round consisted of 20 trials comprising 10 trials formed by positive traits and 10 trials formed by negative traits. The traits in each round were presented in a random and counterbalanced manner across subjects. The trial paradigm is presented in Fig. 1. Each trial started with a fixation cross (jittered randomly for 2 s, 4 s, or 6 s), followed by a positive or negative trait word (each lasting 4 s). After an interval which jittered for 1 s, 3 s, or 5 s, an 11-point self-rating scale was displayed for 11 s. Participants were required to rate the trait using a button placed in their hands within 11 s. Participants underwent sufficient pre-scanning practice using positive and negative traits that were not included in the experimental task sets in order to ensure that they fully understood the instructions and task procedures.

2.3. fMRI data acquisition

fMRI scanning was performed using a 3 T GE DISCOVERY MR750 scanner (General Electric, Fairfield Connecticut, USA) with an eight-channel prototype quadrature birdcage head coil. Functional imaging data were acquired using an echo planar imaging sequence with repetition time/echo time = 2000/30 ms, matrix size = 64 × 64, flip angle = 90°, field of view = 256 mm × 256 mm, voxel size = 3.75 mm × 3.75 mm × 3.2 mm, slices = 43, slice thickness = 3.2 mm, no gap, and 230 volumes per run.

2.4. Data preprocessing

Preprocessing steps and functional imaging data analysis were implemented in the DPABI toolbox (http://rfmri.org/dpabi). The first five time points for each functional image of each run were discarded to permit scanner equilibration. Slice timing correction was conducted to remove differences in acquisition times between different slices. Head motion realigning was performed to correct for head motion. Participants who met the criteria of head motion or displacement exceeding 3 mm or head rotation exceeding 3° were excluded from subsequent analysis. Corrected images were normalized to the standard Montreal Neurological Institute EPI template, resampled to a 2 mm × 2 mm × 2 mm resolution, and smoothed using 8-mm isotropic Gaussian kernel (Plichta et al., 2012). Subjects with x, y, or z translations exceeding 3 mm or rotations exceeding 3° in one or more than one scanning run were excluded from further analysis.

2.5. First-level analysis

First level analysis was performed using Statistical Parametric Mapping (SPM8) toolbox (http://www.fil.ion.ucl.ac.uk/spm/). For each individual and session, the two types of stimuli ("positive traits" and "negative traits") were modeled as regressors-of-interest, with onsets

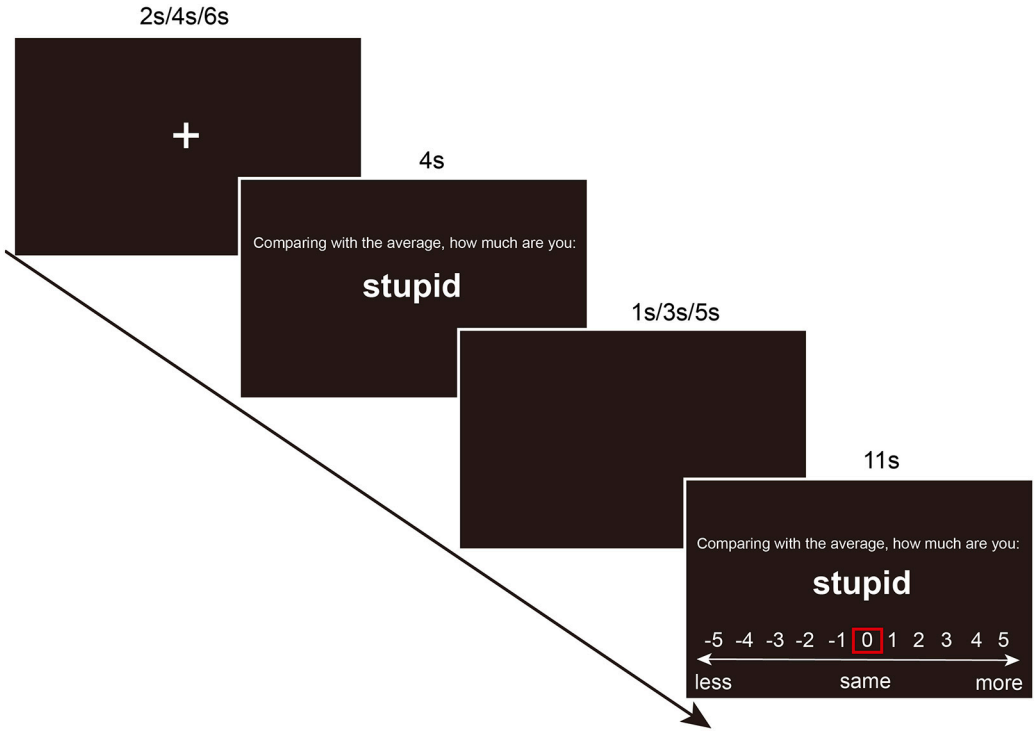

Fig. 1. Schematic example of a trial sequence in the self-related task.

corresponding to the stimulus onset for each trait word. Each regressor was convolved with the canonical hemodynamic response function. The six head motion parameters (regressors of no interest) were included in the design matrix to control for the effects of movement-related artifacts. Then, high-pass filtering (128s cutoff) was performed to remove low-frequency drifts. Individual contrast images for "positive traits" and "negative traits" were entered into second-level analysis to measure group-level significance.

2.6. Second-level analysis

For second-level analysis, a two-way ANOVA computation with a 2 (diagnosis: GAD vs. HC) × 2 (valence: positive traits vs. negative traits) comparison was conducted to examine the main effects of diagnosis and diagnosis-by-valence interaction. Additionally, flexible analysis towards the valence (positive traits vs. negative traits) was performed to detect the main effect of valence. During the two-way ANOVA and flexible analysis, sex, age, and years of education were used as variables of no interest. Gaussian random field (GRF) correction ($p < 0.05$, voxel z value >2.6) was employed as the multiple comparison correction method. Post-hoc comparisons were performed for regions with main or interaction effects.

To evaluate disease-relevant abnormalities in self-related processing, regions showing significant main effect of diagnosis and diagnosis-by-valence interaction were the most interest in subsequent analyses. Aside from self-referential processing (required participants to relate traits to themselves), the self-related task employed in this study may also induce TOM processing (associate given traits with their average peers) and social comparison processing (compare traits with average peers). In this context, for regions showing significant main effect of diagnosis, we not only focus on areas related to self-referential processing, but also areas associated with TOM and social comparison processing. Specifically, the left dorsomedial prefrontal cortex (dmPFC), left inferior frontal gyrus (IFG), and left superior temporal sulcus (STS) were selected as seed regions in subsequent task-related functional connectivity analyses. Of note, for the diagnosis-by-valence interaction of the 2 × 2 ANOVA analysis, only the right postcentral gyrus (PoG) exhibited significant interaction effect. Considering that the interaction effect identified in PoG may simply denote asymmetrical motor preparedness between patients and HCs during the performance of the task. The PoG was not included in the subsequent task-related functional connectivity analysis. Instead, we clearly explained the findings in this area in the discussion section.

2.7. Functional connectivity analysis

Task-dependent functional connectivity between each region of-interest (ROI) derived from the second-level analysis and other brain regions was calculated during processing positive and negative trait words using the psychophysiological interaction (PPI) approach. For each ROI in each individual, a design matrix of PPI analysis was constructed using the following variables: (a) physiological variables: mean ROI time series, (b) psychological variables: onset timings of the experimental variables described in the aforementioned task activation-related first-level analysis, and (c) the interaction term between the physiological and psychological variables. To examine whether functional coupling between the ROI and potential target regions will change when GAD patients processing traits, the contrast images under "positive traits" and "negative traits" were assessed. Contrast images from individuals were then used in subsequent group-level analyses. A 2 × 2 (diagnosis and valence) ANOVA analysis was performed, in which the main effects of diagnosis and diagnosis-by-valence interaction effect were measured. In addition, the flexible analysis was executed to identify the main effect of valence. Gaussian random field (GRF) correction ($p < 0.05$, voxel z value >2.6, GRF-corrected) was conducted to identify the results reached the statistical power.

2.8. Correlations with anxiety symptom

For task-based brain activation analysis, the correlations between the severity of anxiety symptom and functional activation of regions with a significant main effect of diagnosis and diagnosis-by-valence interaction were assessed. Correlations between the severity of anxiety symptom and ROI-based abnormal functional connectivity were evaluated. The statistical threshold of $p < 0.05/N$ (Bonferroni correction) was considered the significance level for correlation analyses, where N was the number of regions with a significant main effect of diagnosis and diagnosis-by-valence interaction.

2.9. Clinical analysis

Medication information of patients was quantified using the total medication load index, a widely used indicator of psychiatric disorders as described in our previous studies (Chen et al., 2020a; Chen et al., 2020b; Cui et al., 2020b; Cui et al., 2020c). The index was calculated based on the criteria proposed by Sackeim (Sackeim, 2001). Each medication was converted to levels 1 to 4 based on dosage and duration, and further coded as absent (0), low dose (1), or high dose (2) based on aforementioned medication levels. Levels 1 and 2 medications were considered low dose and further coded as 1. Levels 3 and 4 medications were considered high dose and further coded as 2. Patients who were not taking any medication were assigned as no-dose subtype. Escitalopram and duloxetine were not included in the criteria of Sackeim and were coded as 0, 1, or 2 according the midpoint of the daily dose range recommended by the Physician's-Desk-Reference. The codes for all medications taken by each patient were added. This final value was assigned as the total medication load index of that patient. The associations of abnormal functional activation and task-based functional connectivity with the total medication load index of patients were calculated (with Bonferroni correction) to assess the effects of medication on neuroimaging findings.

3. Results

3.1. Demographic and clinical characteristics

No significant differences were observed in age, sex, handedness, and head motion between patients with GAD and HCs. A significant between-group difference in years of education was observed. Detailed information is provided in Table 1.

3.2. Self-related behavioral analysis

Self-rating scores for traits, especially negative traits, were lower in patients with GAD than in HCs (Fig. 2). Specifically, significant group difference of the self-rating scores for negative traits ($t_{(69)} = 3.01$, $p < 0.01$) was found between GAD patients (0.29 ± 1.02) and HCs (0.94 ± 0.77).

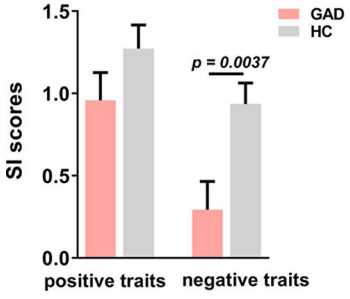

Fig. 2. Between-group differences in self-rating scores (superiority illusion scores).

3.3. Abnormal functional brain activation during processing traits

Results of 2 × 2 ANOVA are presented in Fig. 3 and Table 2. A significant main effect of diagnosis was observed in the left dmPFC, left IFG, left STS, and bilateral lingual gyrus (Fig. 3A). Post-hoc analysis revealed that functional brain activation in these regions during processing traits was significantly reduced in patients with GAD compared to that in HCs ($p < 0.05$, voxel z value >2.6, GRF-corrected). A main effect of valence was observed in regions involved in default and central executive networks (Fig. 3B). With the exception of the bilateral VLPFC and left IPL, other regions exhibited significant increased brain activation during positive trait-processing relative to that during negative trait-processing. A significant diagnosis-by-valence interaction was identified in the right POG (Fig. 3C). Post-hoc analysis of this region revealed significantly decreased brain activation in HCs during processing positive traits compared to that during processing negative traits. Brain activation was greater in patients with GAD than in HCs during processing positive traits.

3.4. Abnormal task-based functional connectivity during processing traits

The left dmPFC, IFG, and left STS were used as seeds during PPI analyses based on their abnormal activation patterns and involvement in self-related and social cognition processing.

No significant main effect of disease, main effect of valence, or diagnosis-by-valence interaction was observed for left dmPFC-based PPI. Significant main effects of diagnosis and valence and a significant diagnosis-by-valence interaction were observed for left IFG-based PPI analysis (Fig. 4A). A significant main effect of diagnosis was observed in the left caudate and putamen. Connectivity between the left IFG and left

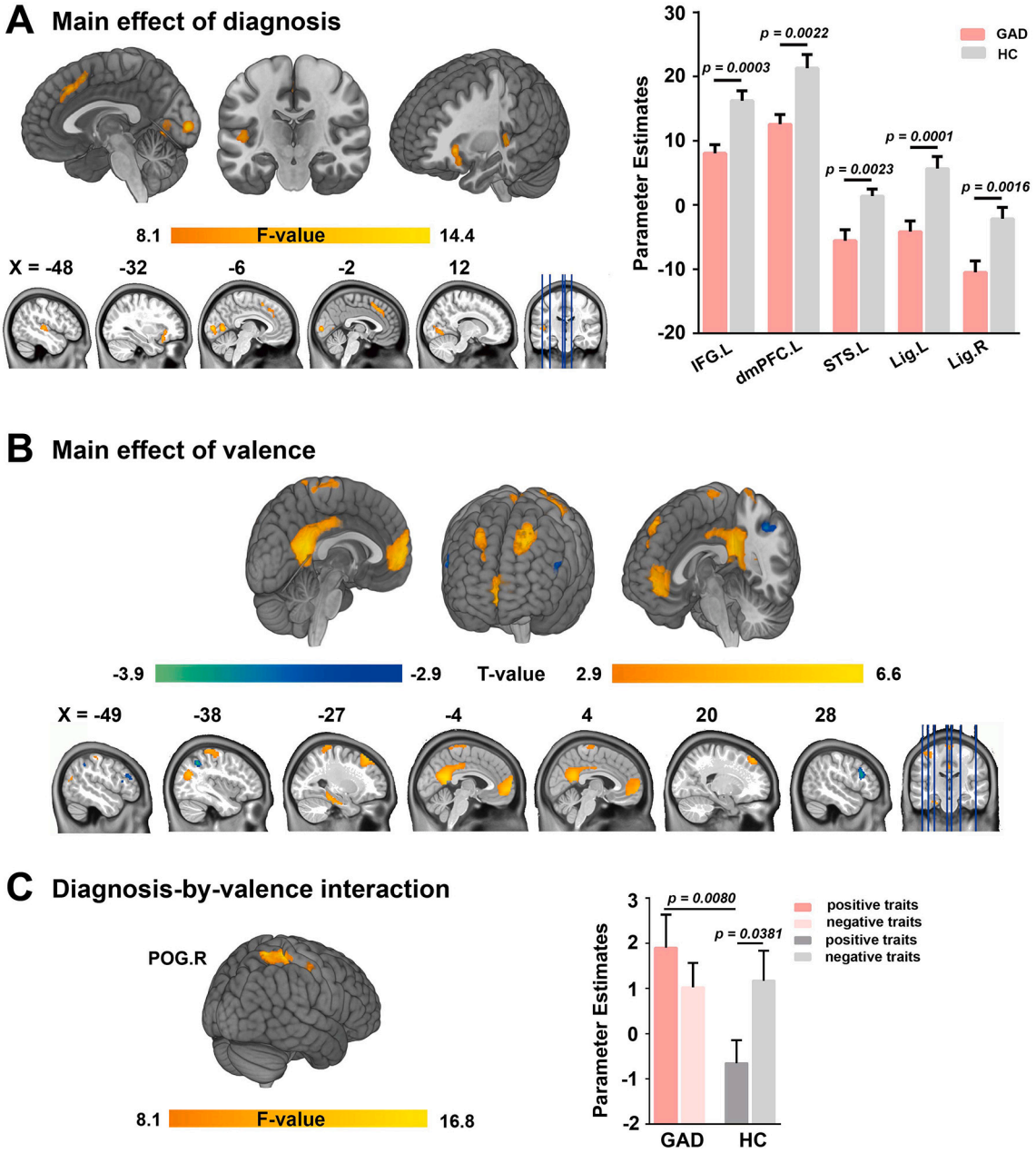

Fig. 3. Brain activation alterations in patients with GAD and corresponding post-hoc analyses during processing of trait words. (A) Significant main effect of diagnosis. (B) Significant main effect of valence. (C) Significant diagnosis-by-valence interaction. GAD, generalized anxiety disorder; IFG, inferior frontal gyrus; dmPFC, dorsomedial prefrontal cortex; STS, superior temporal sulcus; Lig, lingual gyrus; L. left; R, right.

Table 2
Brain regions with significant between-group differences in task activation.

Brain regions	Sphere	Cluster size	Peak	MNI coordinates
	L/R	(voxels)	values	x y z
Main effect of diagnosis			*F-values*	
IFG	L	214	14.4	−36 22–24
dmPFC	L	241	11.3	−2 24 42
STS	L	187	13.0	−44 -26 6
Lingual gyrus	L	754	14.4	−8 -70 4
Lingual gyrus	R	192	10.8	12–70 − 4
Main effect of valence			*T-values*	
MPFC	L/R	1906	5.7	-4 58–2
PCC	L/R	3183	6.5	−6 -56 20
Parahippocampal gyrus	L	486	5.0	−30 -34 -16
Inferior parietal lobule	L	344	−3.9	−36 -54 46
DLPFC	L	1000	6.6	−20 34 50
DLPFC	R	352	5.0	22 40 52
VLPFC	L	435	−3.7	−52 32 26
VLPFC	R	441	−3.7	48 22 24
Middle occipital gyrus	L	677	5.3	−40 -70 26
Postcentral gyrus	L	1508	4.3	−36 -32 68
Diagnosis-by-valence interaction			*F-values*	
Postcentral gyrus	R	972	16.8	46–26 50

IFG, inferior frontal gyrus; dmPFC, dorsalmedial prefrontal cortex; STS, superior temporal sulcus; MPFC, medial prefrontal cortex; PCC, post cingulate cortex; DLPFC, dorsolateral prefrontal cortex; VLPFC, ventrolateral prefrontal cortex; L, left hemisphere; R, right hemisphere.

striatal regions was decreased in GAD patients during positive and negative trait-processing. A main effect of valence was anchored in the bilateral thalamus and right insula extending to the right caudate; the connectivity between these regions and the left IFG was decreased during processing positive traits compared to that during processing negative traits. A significant diagnosis-by-valence interaction was identified in the bilateral caudate and right insula. Significantly increased connectivity of the left IFG with bilateral caudate and right insula was observed in HCs but not GAD patients during processing negative traits compared to that during processing positive traits. Compared to HCs, patients with GAD exhibited significantly weaker connectivity between the left IFG and regions of bilateral caudated and right insula during processing negative traits.

STS-based PPI results are presented in Fig. 4B. A main effect of diagnosis was anchored in the caudate and insula clusters. Compared to HCs, patients with GAD exhibited decreased functional connectivity between the STS and these two clusters during processing traits. A main effect of valence was observed in the right dorsolateral prefrontal cortex (DLPFC). Connectivity between the right DLPFC and left STS was greater during processing positive traits than during processing negative traits. A diagnosis-by-valence interaction was observed in the right angular gyrus and precuneus. The connectivity between these regions and the left STS was decreased in HCs during processing positive traits compared to that during processing negative traits. Conversely, GAD patients exhibited increased connectivity of the left STS with right angular and precuneus during processing positive traits compared to that during processing negative traits.

3.5. Correlations with behavior and anxiety symptom

No significant correlations were observed between the severity of anxiety symptom and abnormal functional activation of regions with a significant main effect of diagnosis and diagnosis-by-valence interaction during the self-related task. Aberrant task-based functional connectivity between the left STS and left insula exhibited marginally significant negative correlation with HAMA scores of patients ($r = -0.4050$, $p = 0.0158$, uncorrected) (Supplementary Fig. 1). Although this correlation was not passed the multiple correlation comparison, it may provide potential clues in investigating the association between anxiety symptom and brain dysfunction of GAD.

3.6. Correlations with total medication load index

No significant associations were observed between either abnormal brain activation or abnormal task-based functional connectivity and total medication load index in patients with GAD.

4. Discussion

This study demonstrated aberrant self-related processing in patients with GAD. This manifested as lower self-rating scores particularly for negative traits, suggestive of negative self-bias in patients with GAD. Decreased brain activation in the dmPFC, IFG, STS, and lingual gyrus was observed in GAD patients compared to that in HCs during processing traits. Connectivity between the IFG and basal ganglia, and that between the STS and regions involved in reward and emotional processing, were attenuated in GAD during processing traits. Connectivity between the IFG and emotion-related regions was stronger during processing negative traits than during processing positive traits in HCs but not in GAD patients. Connectivity of the IFG with basal ganglia and insula was decreased during processing negative traits in GAD patients compared to that in HCs. Connectivity within regions associated with TOM processing was increased in HCs but decreased in GAD patients during processing negative traits compared to that during processing positive traits. These findings provide mechanistic insight into the neural basis of abnormal self-related processing and social cognition in GAD patients.

During the processing of positive and negative traits, regions linked to self-related processing, cognitive control, and TOM processing, such as the dmPFC, IFG, and the STS, exhibited reduced activation in patients with GAD. For HCs, positive traits were generally more likely to be considered as self-related (Lemogne et al., 2012), and have processing priority, so as to help healthy individuals maintain a positive self-concept (Sedikides and Green, 2000). The MPFC and posterior cingulate cortex were activated in HCs during self-evaluation of personality traits (Beer and Hughes, 2010), and greater activation in the dmPFC was observed in positive self-related scenarios (Fields et al., 2019). These findings in HCs suggest an important role of the dmPFC in positive self-views. Decreased dmPFC activation implies impairments in positive bias in the self-concept of GAD. These findings were consistent with the behavioral observations demonstrating that GAD patients exhibited a weaker superiority illusion, especially for negative traits. Evidence for the involvement of the IFG in emotional regulation manifested as greater activation in HCs during emotional processing (Camacho et al., 2019, Goldin et al., 2008). Given that NSRP is a core factor sustaining negative emotion in anxiety disorder (Dixon et al., 2020), inordinate NSRP in GAD may further impacting its emotion regulation ability, manifested as IFG hypoactivation. Additionally, patients with GAD exhibited weaker activation in the STS. The STS is a key node involved in TOM processing, which enables individuals to infer the emotions, intentions, mental states, and beliefs of others (Abu-Akel and Shamay-Tsoory, 2011). Positive association between greater SRP and greater TOM accuracy has been found in healthy population (Dinulescu et al., 2021). To date, studies investigating TOM in GAD patients have been limited to behavioral observations (Zainal and Newman, 2018), and neuroimaging evidence is lacking. Although the explicit role of the STS in TOM processing could not be identified herein, decreased activity in the STS in GAD patients may have affected TOM processing in patients and may be associated with distorted inferences regarding traits relative to those of average peers, and may affect the SRP in GAD. The diagnosis-by-valence interaction observed in the right PoG suggests asymmetrical motor preparedness between patients and HCs. Specifically, HCs exhibited a trend for rightward motor preparedness, indicative of a positive bias,

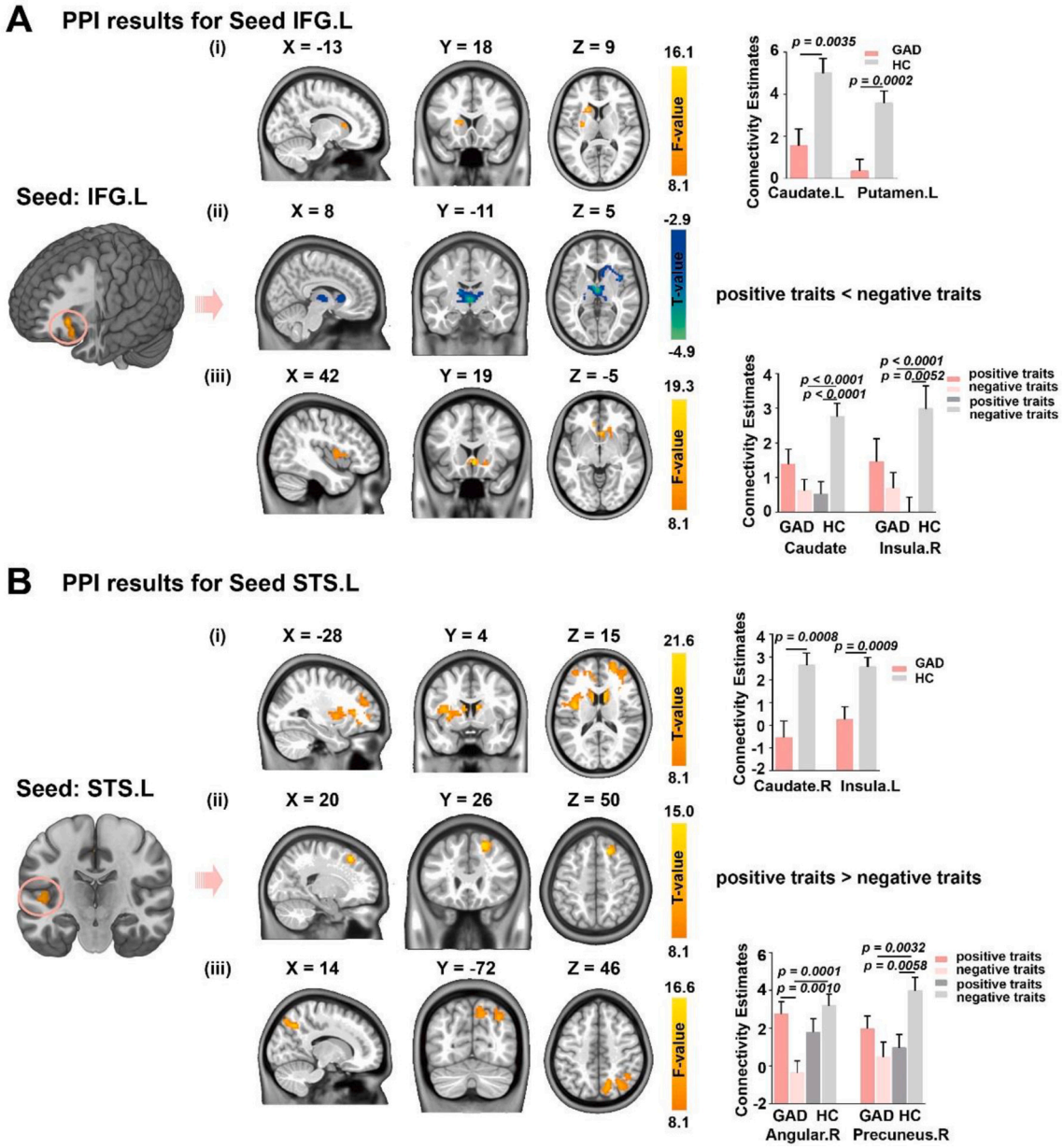

Fig. 4. Results for the left IFG- and STS-based PPI analyses. (A) Results for the left IFG-based PPI computation. (B) Results for the left STS-based PPI analysis. (i), (ii), and (iii) in (A) and (B) separately represent the main effect of diagnosis, main effect of valence, and diagnosis-by-valence interaction effect. IFG, inferior frontal gyrus; STS, superior temporal sulcus; PPI, psychophysiological interaction; DLPFC, dorsolateral prefrontal cortex; L, left; R, right.

whereas GAD patients demonstrated a leftward motor preparedness, indicative of a negative bias, when positive traits displayed. Conversely, HCs exhibited a leftward motor preparedness and GAD patients demonstrated a rightward motor preparedness when presented with negative traits.

Our findings provide insight into the configuration of abnormal networks involving the dmPFC, IFG, and STS. Compared to HCs, GAD patients exhibited significantly decreased connectivity between the IFG and basal ganglia, suggesting excessive NSRP coupled with weaker emotional regulation in GAD patients during processing traits. The diagnosis-by-valence interaction indicated that connectivity of the IFG with reward and emotion-related regions was strengthened in HCs during processing negative traits compared to that processing positive traits, suggesting that HCs recruited more emotional regulation for negative stimuli to avoid triggering maladaptive cognitive reactivity from negative self-evaluation. Decreased connectivity between the PFC with cortical and subcortical regions such as the insula (Cui et al., 2020a) and amygdala (Makovac et al., 2016; Roy et al., 2013) have been reported in GAD patients. This highlights impairments in emotional regulation and interoceptive awareness in GAD patients (Mochcoyitch et al., 2014). In this study, connectivity of the IFG with reward and emotion-related regions was decreased in GAD patients during processing negative traits compared to that in HCs, indicating impaired emotional regulation of traits, especially those with negative valence. These findings agree with previous reports that GAD patients have poor regulatory skills for negative emotions (Ball et al., 2013, Mennin et al., 2005) which may be associated with negative self-evaluation.

Greater connectivity was noted between the STS and DLPFC in participants during processing positive traits compared to that processing negative traits. The STS is a key node linked to TOM processing, which is selectively activated during computations of mental states of others (Abu-Akel and Shamay-Tsoory, 2011) and contributes to social

cognition (Allison et al., 2000). The DLPFC is associated with cognitive TOM (Abu-Akel and Shamay-Tsoory, 2011). A possible explanation for the enhanced connectivity between these regions is that positive traits engaged more TOM processing compared to negative traits. The angular gyrus and precuneus also critical for TOM processing (Mukerji et al., 2019) and were identified in subsequent diagnosis-by-valence interaction analyses. During processing negative traits, the connectivity of the STS with angular gyrus and precuneus was increased in HCs but attenuated in GAD patients. Further, HCs exhibited increased connectivity whereas GAD patients demonstrated decreased connectivity in regions correlated with TOM during processing negative traits compared to that processing positive traits. These findings highlight the dichotomy between HCs and GAD patients in associating negative traits with others.

Attenuated connectivity of the STS with the caudate and insula was observed in GAD patients during processing traits. The caudate and insula are associated with reward and emotion (Haynos et al., 2021, Hua et al., 2020). The task in this study involved multiple processes including SRP, TOM processing, and social comparison. Due to widespread positively biased SRP in the general population, social comparisons may be perceived as rewarding for individuals with superiority illusion. However, patients with GAD may not perceive social comparisons as rewarding. This may explain the weaker connectivity of the STS with reward and emotion-related regions observed in GAD patients during processing traits. Additionally, functional connectivity between the STS and insula was marginally significantly negatively correlated with HAMA scores of GAD patients, implying that anxiety symptoms were aggravated in relation to the weaker correlation between these regions. Notably, the correlation did not reached the statistical significance, which may resulted from several reasons, such as relative small samples and medical treatment of patients. Although the correlation we observed is just marginally significant, it may provide us with important clues in investigating brain dysfunction in GAD patients in the future.

Several limitations of this study should be addressed. First, the findings in this study may have been insufficiently powered due to relatively loose statistical corrections for small samples, which should be further verified in our future studies using larger samples and strict correction methodologies. Second, although the unmatched variable, years of education, was regressed as a covariate during neuroimaging analyses, confounding effects of education cannot be excluded. Third, most of the patients were treated with medications, and confounding effects of medications cannot be excluded. Nevertheless, no significant associations between medication information and neuroimaging findings were identified. In this context, the current results should be verified using medication-free samples in the future. Finally, the marginally significant correlation between anxiety symptom of GAD and its altered self-referential processing-related functional connectivity should be further validated through larger samples in the future.

5. Conclusion

In conclusion, patients with GAD exhibited impaired self-related processing regarding their traits, especially those with negative valence. The neuroimaging results indicated that patients with GAD demonstrated aberrant brain function in regions supporting self-related processing, cognitive control, and TOM processing during processing traits. Furthermore, aberrant functional organization in these regions as noted, which manifested as altered functional connectivity of reward and emotion-related regions with the PFC and STS, and abnormal connectivity within the regions linked to TOM ability during processing traits. Collectively, these findings provide insight into the neuropathology underscoring distorted self-related processing in GAD patients and pave the way for developing neural intervention-based treatments for this condition.

Author Statement

Qian Cui and Huafu Chen conceptualization, data curation, funding acquisition, project administration, supervision. Qian Cui writing- review. Yuyan Chen, formal analysis, writing-original draft. Qin Tang, Wei Sheng, and Di Li investigation, methodology. Yuhong Zeng and Kexing Jiang visualization. Zongling He and Huafu Chen validation, editing.

Ethical statement

This study was approved by the ethical committee of the University of Electronic Science and Technology of China and is listed on Clinical-Trials.gov (Registration Number: NCT02888509). All participants provided written informed consent prior to participation.

Declaration of Competing Interest

All authors declare that they have no conflicts of interest.

Data availability

The authors do not have permission to share data.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pnpbp.2022.110595.

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