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RESEARCH ARTICLE

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Insula shows abnormal task-evoked and resting-state activity in first-episode drug-naïve generalized anxiety disorder

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Abstract

Background: Interoception is associated with neural activity in the insula of healthy humans. On the basis of the somatic symptoms in generalized anxiety disorder (GAD), especially abnormal heartbeat perception, we hypothesized that abnormal activity in the insula was associated with interoceptive awareness in patients with GAD.

Methods: We investigated the psychological correlates of interoceptive awareness in a sample of 34 patients with first-onset, drug-naïve GAD and 30 healthy controls (HCs). Furthermore, we compared blood oxygenation level-dependent responses between the two groups during a heartbeat perception task to assess task-evoked activity and its relationship with psychological measures. We also examined between-group differences in insular subregions resting-state functional connectivity (rsFC), and its relationship with anxiety severity.

Results: Patients with GAD had significantly higher body perception scores than HCs. They also exhibited greater task-evoked activity in the left anterior insula, left posterior insula, and right anterior insula during interoceptive awareness than HCs. Left anterior insula activity was positively correlated with body awareness in patients with GAD, and rsFC between the left anterior insula and left medial prefrontal gyrus was negatively correlated with somatic anxiety severity.

Conclusions: Investigating a sample of first-episode, drug-naïve patients, our study demonstrated abnormal interoceptive awareness in patients with GAD and that

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this was related to abnormal anterior insular activity during both rest and task. These results shed new light on the psychological and neural substrates of somatic symptoms in GAD, and they may serve to establish abnormal interoceptive awareness as a neural and psychological marker of GAD.

KEYWORDS
anxiety, awareness, functional connectivity, functional magnetic resonance imaging (fMRI), generalized anxiety disorder, insula, interoception

1 | INTRODUCTION

Generalized anxiety disorder (GAD) is characterized by chronic, excessive anxiety and worry accompanied by somatic symptoms such as restlessness, muscle tension, palpitation, cardiopalmus, irritability, and sleep disturbance. The disorder has a lifetime prevalence of ~5% and is associated with a significant personal and economic burden (Kujanpaa, Jokelainen, Auvinen, & Timonen, 2017; Munir & Hughes, 2018). Some behavioral and psychophysiological studies have reported abnormal interoceptive awareness in patients with an anxiety disorder (Andor, Gerlach, & Rist, 2008; Chan et al., 2015; Chan, von Leupoldt, Liu, & Hsu, 2014; Ehlers & Breuer, 1992; Grossi et al., 2017; Hoehn-Saric, McLeod, & Zimmerli, 1989; Hu et al., 2012), which has led to theories that anxiety disorders are fundamentally disorders of interoception (Domschke, Stevens, Pfleiderer, & Gerlach, 2010; Khalsa et al., 2018; Mallorqui-Bague, Bulbena, Pailhez, Garfinkel, & Critchley, 2016). We recently found that heartbeat evoked potential is marginally higher in patients with GAD than in healthy controls (HCs; Pang et al., 2019), suggesting that GAD involves deficient adaptation to interoceptive signals. Nonetheless, the exact psychological and neural substrates of abnormal interoception in GAD remain poorly understood.

Behaviorally, interoceptive awareness in patients with GAD was investigated in two recent studies. One investigation by Ehlers and Breuer (1992) used the heartbeat perception score of the Mental Tracking Task as the primary metric of interoceptive awareness in patients with GAD. They reported greater heartbeat perception in patients with panic disorder and GAD than in those with major depression. In contrast, Hu et al. (2012) demonstrated that only patients with panic disorder had significantly greater heartbeat perception than HCs, whereas those with GAD did not. Thus, the role of interoception in GAD remains poorly understood, so the first goal of the present study was to explore interoceptive awareness at the behavioral and psychological levels in patients with GAD. To this end, we recruited a sample of patients with first-episode, drug-naïve GAD. We used the Body Perception Questionnaire (BPQ) to measure the patients' interoceptive awareness.

Recent functional neuroimaging studies and meta-analyses have shown that the insula is an important locus for interoceptive awareness in healthy individuals (Avery et al., 2017; Hassanpour et al., 2016; Hassanpour et al., 2018; Salomon et al., 2018; Schulz, 2016; Tan et al., 2018; Wiebking et al., 2010; Wiebking, Duncan, Qin et al., 2014; Wiebking, Duncan, Tiret, et al., 2014). Usually, the insula includes two subregions: the anterior and posterior insula (Gasquoine, 2014). The anterior insula likely plays a significant role in interoceptive awareness of the physical self as a sentient entity (Craig, 2009; Domschke et al., 2010; Seth, 2013). Activation of the anterior insula when focusing on cardiac interoception has been positively correlated with state and trait anxiety levels in healthy volunteers (Tan et al., 2018). Moreover, Caseras et al. (2013) reported that increased anterior insular activity was associated with interoceptive awareness in patients with the phobia. However, the anterior insula and its relationship to interoceptive awareness have not yet been investigated in patients with GAD. Therefore, the second goal of the present study was to investigate anterior insular activity during both interoceptive awareness in task-evoked activity and resting state in patients with GAD.

Many studies have suggested that GAD is related to dysregulated cognitive processing such as decision making and affective processes and that the disorder involves impaired connections among various regions, including the insula, prefrontal cortex (PFC), amygdala, putamen, striatum, and cingulate cortex (Assaf et al., 2018; Krain et al., 2006; White et al., 2017). Moreover, the left anterior insula (the seed-region under experimental task "anxiety condition") exhibits functional connectivity (FC) to some regions during rest, including the left caudate, inferior PFC, dorsal parietal cortex, and midline supplementary motor area (SMA; Simmons et al., 2013). This provides more indirect evidence that anterior insula plays a role in GAD. It follows that as the insula plays a role in interoceptive awareness (see above), the abnormal interoceptive awareness seen in patients with GAD may be related to abnormal insular activity during both rest and task-evoked activity.

The current study was conducted to clarify the neural correlates of interoceptive symptoms in a sample of patients with first-episode, drug-naïve GAD. To this end, participants were subjected to functional magnetic resonance imaging (fMRI) during a well-validated heartbeat counting task, in which participants focused on the sensation of their heartbeat (Wiebking & Northoff, 2015; Wiebking et al., 2010; Wiebking, Duncan, Qin et al., 2014; Wiebking, Duncan, Tiret, et al., 2014). In addition, the behavioral level of interoceptive awareness was measured using the BPQ. On the basis of prior studies (Caseras et al., 2013; Domschke et al., 2010; Ehlers & Breuer, 1992; Simmons et al., 2013), we hypothesized that increased interoceptive awareness at the behavioral level, as well as at the level of neural activity within the anterior insula, underlies persistent pathological anxiety (e.g., patients' somatic awareness and anxiety severity). We further hypothesized that activity in the anterior insula during both task-evoked activity and rest would be correlated with patients' self-reported somatic awareness (BPQ) and behavioral measures of anxiety severity (HAMA).

2 | METHODS AND MATERIALS

2.1 | Ethics statement

The present study was approved by the Research Ethics Committee of Shanghai Mental Health Center, Shanghai, China. The study was in line with the Declaration of Helsinki. All participants provided written informed consent.

2.2 | Participants and procedures

Thirty-four patients with GAD and 30 healthy controls (HCs) were recruited from September 2014 to May 2016 at our center. All patients with GAD were seeking help for the first time and were drug-naïve upon study entry. One expert psychiatrist confirmed the diagnosis according to the criteria of the *Diagnostic and Statistical Manual of Mental Disorders*, *4th ed.* (*DSM-IV*); later, two research doctors further checked the patients' diagnosis using the Chinese version of the Mini International Neuropsychiatric Interview (MINI; Si et al., 2009). Patients with both GAD and comorbid disease were excluded. The inclusion criteria were as follows: (a) age ranging from 18 to 50 years, (b) ≥6 years of education, (c) first-episode, drug-naïve GAD, (d) Hamilton Anxiety Scale (HAMA; Hamilton, 1959) score ≥14, and (e) Hamilton Depression Scale (HAMD; Hamilton, 1960) score <14. The exclusion criteria were as follows: (a) intellectual disability, dementia, and/or other neurological illness, (b) history of head trauma leading to loss of consciousness, (c) current severe somatic disease, such as cancer, heart failure, or pneumonia, (d) current substance abuse or dependence, (e) presence/history of psychotic disorders, (f) contraindication to magnetic resonance imaging (MRI), and (g) reported previous episode of GAD.

We recruited HCs from the local area via poster advertisements. In these participants, we used the MINI to screen for current or past mental disorders. The inclusion and exclusion criteria for HCs were identical to those for patients with GAD, except for the presence of GAD. The HCs were matched closely to the GAD group members for age, gender, body mass index, estimated intelligence quotient (IQ), and education duration.

All participants were administered the HAMA, 7-item Generalized Anxiety Disorder Scale (GAD7), HAMD, BPQ, and IQ estimation before brain scanning. Anxiety symptoms were assessed by clinicians using the HAMA, which has a psychic anxiety subscale and a somatic anxiety subscale. Anxiety symptoms were also assessed using the self-report scale GAD7 (Mossman et al., 2017). IQ scores were assessed using the Revised Chinese version of the Wechsler Abbreviated Scale of Intelligence (Gong & Dai, 1984). Body perceptions were assessed using the BPQ, which is a translation of the original version developed by Porges (1993). The BPQ included several subscales: The awareness subscale comprised 45 items that assessed the participants' awareness regarding their body processes; The stress response subscale comprised 10 items evaluating the participants' awareness of their perceived bodily changes due to stress when in an imagined stressful situation; The autonomic nervous system reactivity (ANSR) subscale included 27 items assessing the participants' own autonomous nervous system reactions; The stress style subscale (style 1 and style 2) contained 12 items evaluating how participants responded to stress. Higher BPQ scores indicated greater self-reported autonomic reactivity and awareness.

The data of two patients with GAD were excluded from the analyses because they showed excessive head movement during MRI. Thus, 32 patients with GAD and 30 HCs were included in the final analyses. The participants' demographic characteristics are listed in Table 1.

2.3 | Task design

We used E-Prime 2.0 version (Psychology Software Tools Inc.) to design and administer the experiment. We used fMRI Hardware System (IFIS-SA; Invivo Corporation, Orlando, FL) to project the task stimuli from a liquid-crystal display projector to the screen. The stimuli were visible through an adjustable mirror located on the head coil, which was angled at 45° to the participant's eye line.

The event-related fMRI design used to investigate interoceptive and exteroceptive awareness was based on the paradigm of Critchley and Pollatos' (Critchley, Wiens, Rotshtein, Ohman, & Dolan, 2004; Pollatos, Schandry, Auer, & Kaufmann, 2007), which was further modified by Wiebking et al. (Wiebking & Northoff, 2015; Wiebking et al., 2010; Wiebking, Duncan, Qin, et al., 2014; Wiebking, Duncan, Tiret, et al., 2014). The entire experiment included four scanning runs of 9.6 min, for a total scanning time of 38.4 min. Each scanning run consisted of three independent conditions—a rest period, an interoceptive task, and an exteroceptive task-each of which was applied 48 times in a pseudorandom sequence. At the beginning of each scanning session, participants were instructed to listen to an auditory tone and adjust its volume until it reached the same perception level as their heartbeat. During the interoceptive condition, a black heart was presented on a light background for 9–13 s, and the participants were instructed to silently count their own heartbeats. During the exteroceptive condition, a black musical note was presented on a light background for 9–13 s; the participants were then instructed to listen to the tones that were played through the scanner loudspeaker and to silently count the number of tones. During the rest condition, a black cross was displayed on a light background for 9–13 s; the participants were then instructed to maintain a relaxed state and to reduce any thinking. These rest conditions served as the baseline.

TABLE 1 Demographic and clinical characteristics

	HC	GAD	*t*	*p*	Cohen's *d*
Sample size (*n*)	30	32	–	–	–
Age (years)	31.0 (6.4)	33.1 (8.3)	1.086	.282	−0.28
Gender (Male/Female)	17/13	21/11	χ^2 = 0.524	.603	–
Educated years	13.5 (2.4)	14.0 (2.1)	0.875	.385	−0.22
Body Mass Index (kg/m^2)	23.2 (3.5)	21.8 (3.1)	−1.642	.106	0.42
Estimate IQ	105.6 (11.6)	103.2 (10.1)	−0.868	.389	0.22
HAMA-somatic	0.2 (0.5)	8.7 (3.5)	13.501	<.001	−3.4
HAMA-psychic	0.7 (1.1)	12.5 (2.7)	23.034	<.001	−5.7
HAMD	0.3 (0.7)	10.4 (3.1)	17.844	<.001	−4.5
GAD7	2.1 (2.9)	12.9 (4.0)	12.101	<.001	−3.1
BPQ-total scores	166.9 (31.8)	220.6 (35.0)	6.304	<.001	−1.6
BPQ-awareness	61.8 (13.5)	86.2 (17.5)	6.124	<.001	−1.6
BPQ-stress response	16.2 (6.6)	26.2 (6.6)	5.959	<.001	−1.5
BPQ-ANSR	34.3 (7.6)	43.5 (11.3)	3.734	<.001	−1.0
BPQ-stress style1	20.0 (4.8)	24.8 (3.8)	4.367	<.001	−1.1
BPQ-stress style2	5.2 (2.0)	7.2 (2.5)	3.369	.001	−0.9

Note: Subjects demographics and clinical data were presented as mean (standard deviation) for quantitative data.
Abbreviations: ANSR, autonomic nervous system reactivity; BPQ, Body Perception Questionnaire; GAD, generalized anxiety disorder; HAMA, Hamilton Anxiety Scale; HAMD, Hamilton Depression Scale; HC, healthy control; IQ, intelligence quotient.

2.4 | fMRI data acquisition

All images were acquired on a 3.0-T Siemens MAGNETOM Verio syngo MR B17 scanner equipped with a 12-channel head coil (Siemens, Erlangen, Germany). Head motion was limited using foam padding and scanner noise was reduced using earplugs. The parameters for sagittal three-dimensional T1-weighted images were as follows: repetition time (TR), 1,900 ms; echo time (TE), 2.46 ms; inversion time (TI), 900 ms; flip angle (FA), 9°; field of view (FOV), 256 × 256 mm; matrix, 256 × 256; slice thickness, 1 mm (no gap); and 192 sagittal slices.

Four echo-planar imaging (EPI) scans were obtained for task fMRI and one EPI scan was obtained for rest fMRI. The sequence parameters were as follows: TR, 2,000 ms; TE, 32 ms; FA, 70°; FOV, 240 × 240 mm; matrix, 64 × 64; slice thickness, 5 mm; 30 interleaved transverse slices; voxel size, 3.8 × 3.8 × 5 mm.

2.5 | Task data analysis

The task fMRI data were preprocessed using SPM12 (http://www.fil.ion.ucl.ac.uk/spm). The 247 volumes of each run were corrected for the time delay between different slices, and all volumes were realigned to the first volume. Head motion parameters were computed in terms of the estimated translation in each direction and the angular rotation of each volume on each axis. Each participant had a maximum displacement of <3 mm in any cardinal direction (*x*, *y*, *z*) and a maximum spin (*x*, *y*, *z*) of <3°. The T1-weighted images were linearly co-registered to the corresponding mean functional image; the transformed T1-weighted images were then segmented into gray matter, white matter, and cerebrospinal fluid. The gray matter maps were linearly co-registered to the tissue probability maps in Montreal Neurological Institute space. The motion-corrected functional volumes were spatially normalized to the individual's T1-weighted image using the parameters estimated during linear co-registration. The functional images were resampled into 3 × 3 × 3 mm voxels. Finally, all datasets were smoothed with a Gaussian kernel of 8 × 8 × 8 mm full-width at half maximum.

At the single-subject level, three regressors of interest (fixation, heartbeats, and pure tones) were modeled using SPM12 and convolved with the canonical hemodynamic response function. The voxel time series were high-pass filtered at 1/128 Hz to account for non-physiological slow drifts in the measured signal. They were then modeled for temporal autocorrelation across scans with an auto-regressive model.

2.6 | Resting fMRI preprocessing and FC calculation

The resting-state fMRI data were preprocessed using SPM12 and DPABI (V3.1; http://rfmri.org/dpabi). The first 10 volumes of these data in each participant were discarded to ensure that the fMRI signal had reached equilibrium and that the participants had adapted to the scanner noise. The remaining 155 volumes were then corrected for the acquisition time delay between slices. Derived realignment was used to correct potential rigid head motion with thresholds (translational or rotational motion parameters lower than 3 mm or 3°). We also calculated frame-wise displacement (FD) to reflect the mismatch between volume and volume head position (Power, 2017; Power, Barnes, Snyder, Schlaggar, & Petersen, 2013; Power, Schlaggar, & Petersen, 2015). The FD was obtained from the derivatives of the rigid-body realignment estimates. We deleted spike volumes, as well as the volumes immediately before and after them when the FD of the specific volume exceeded 0.5. Several nuisance covariates were regressed out from the data, namely, six motion parameters and their first temporal derivatives, as well as the average signals of ventricular and white matter. A band-pass filter with a frequency from 0.01 to 0.08 Hz was applied after this. The following normalized and smoothing steps were the same as those used to process the task fMRI data.

Seed-based FC was calculated using the REST toolbox (V1.8; http://restfmri.net). Seeds were defined as regions showing abnormal activation in the heartbeats > pure tones contrast. For heartbeats > pure tones contrast, the patients with GAD demonstrated elevated activity in several regions, the insula were identified using Neuromorphometric atlas and then selected as masks to carry out seed-based FC.

2.7 | Statistical analysis

To analyze task data, we conducted the following three two-sample t tests to identify the main effects in SPM12: heartbeats > fixation, pure tones > fixation, and heartbeats > pure tones. The main effects measured in the above contrasts were compared between HCs and patients with GAD, with age and gender as covariates.

To analyze FC, a two-sample t test was performed to test for group differences in seed-based FC between HCs and patients with GAD, with age and gender as covariates.

To analyze task fMRI and FC, we used the Monte Carlo simulation (AlphaSim program in the REST toolbox) to perform multiple comparisons with a correction threshold of $p < .05$ (single-voxel $p = .002$; cluster connection radius [r] = 5 mm; 5,000 simulations). The high initial voxel threshold was chosen based on the report of Woo, Krishnan, & Wager, (2014) to minimize false positives and account for smaller, but significant, activation clusters.

Demographic and clinical data were analyzed using SPSS version 19.0 (SPSS Inc., Chicago, IL). We used t tests for continuous variables and χ^2 tests for categorical variables. Differences were considered statistically significant at $p < .05$. The effect size (d) was calculated using Cohen's formula. Correlations were analyzed between anxiety scores (HAMA-somatic subscale, HAMA-psychic subscale, each with 14 items in the HAMA and GAD7) and BPQ (total score and five subscores) in patients with GAD. Due to the BPQ-awareness and HAMA were discrete scores, the data of neuroimaging was the β value which was real-valued, so Spearman's correlation analysis was employed, with a Bonferroni correction for two comparisons ($p < .05/2$ or $p < .025$).

3 | RESULTS

3.1 | Participant characteristics and BPQ scores

Patients with GAD and HCs did not significantly differ in age, gender, education level, body mass index, or IQ. All participants were right-handed. As expected, the GAD group scored significantly higher than the HCs on the HAMA subscales (psychic anxiety and somatic anxiety), GAD7, and BPQ-total score, and five BPQ subscale scores (Table 1 and Figure 1).

Among patients with GAD, GAD7 was significantly correlated with the total BPQ score (Spearman's $\rho = 0.506$; $p < .01$), BPQ-awareness score ($\rho = 0.551$; $p < .01$), and BPQ-ANSR ($\rho = 0.432$; $p < .05$). Moreover, the BPQ-awareness score and total BPQ score were both significantly correlated with respiratory symptoms in the HAMA-somatic subscale ($\rho = 0.530$ for BPQ-awareness score and 0.467 for BPQ-total score; $p < .01$).

3.2 | Task-related activity

Patients with GAD were compared with HCs in terms of the three abovementioned contrasts. For the heartbeats > fixation contrast, patients with GAD exhibited greater activity in the left anterior insula (AlphaSim-corrected $p < .05$) than HCs

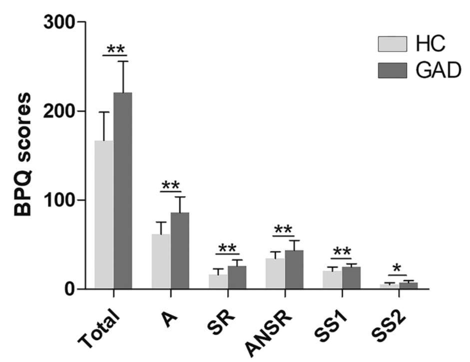

FIGURE 1 Results of the Body Perception Questionnaire (BPQ) in healthy controls (HCs) and patients with GAD (means ± standad deviation). A, awareness; ANSR, autonomic nervous system reactivity; GAD, generalized anxiety disorder; SR, stress response; SS1, stress 1; SS2, stress 2; Total, total score; *$p < .001$; *$p < .05$

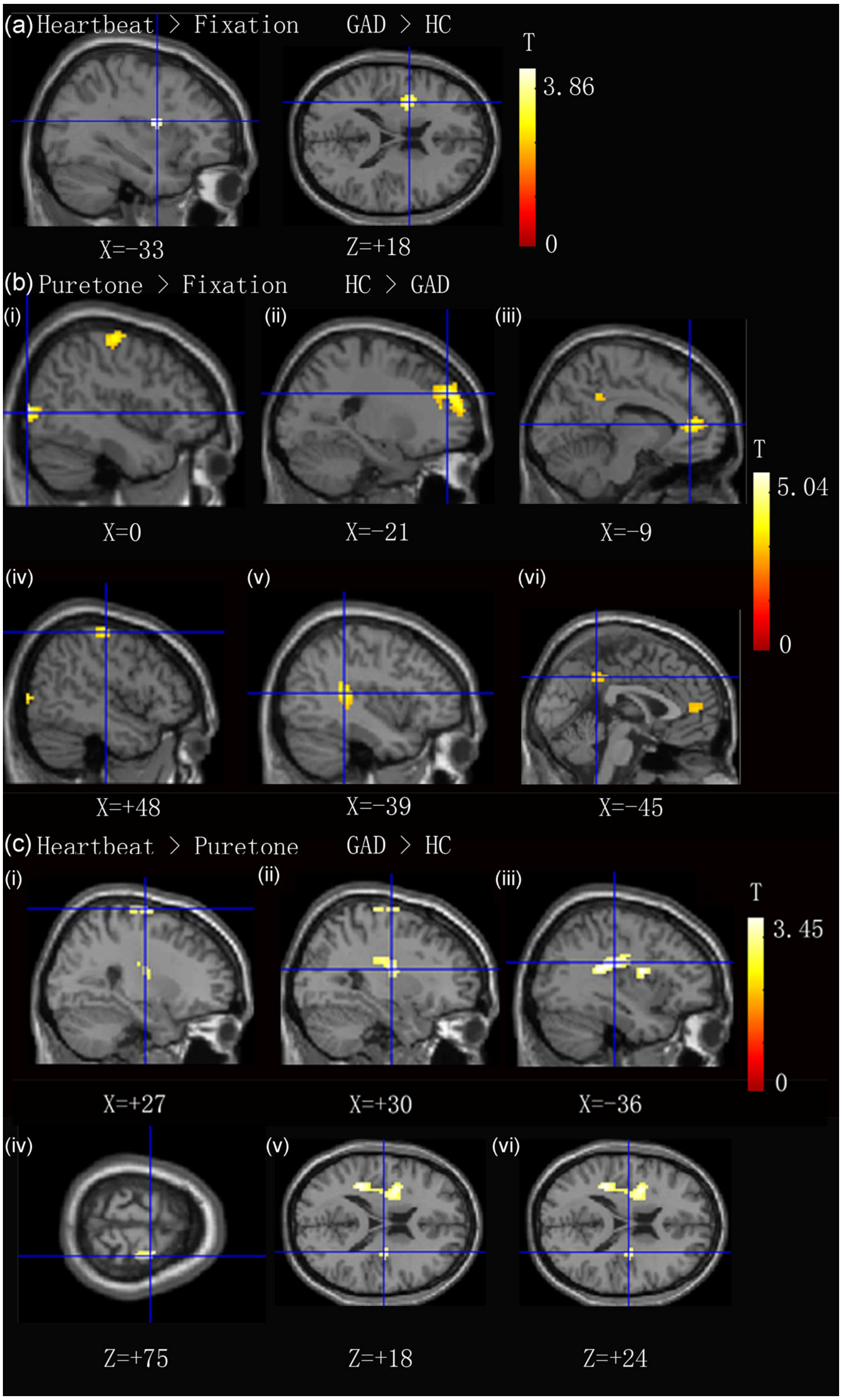

FIGURE 2 (a) In the heartbeats > fixation contrast, patients with GAD showed greater activity in the left anterior insula ($p < .05$, corrected) than HCs. (b) In the pure tones > fixation contrast, patients with GAD showed reduced activity in the right occipital gyrus (i), left superior frontal gyrus (ii), left anterior cingulate gyrus (iii), right precentral gyrus (iv), left superior temporal gyrus, and precuneus (vi); $p < .05$, corrected) than HCs. (c) In the heartbeats > pure tones contrast, patients with GAD showed greater activity in the right precentral cortex (i & iv), right anterior insula (ii & v), and left anterior and posterior insula (iii & vi); $p < .05$, corrected, than HCs. GAD, generalized anxiety disorder; HC, healthy control

(Figure 2a and Table 2). For the pure tones > fixation contrast, patients with GAD demonstrated lower activity in the left superior frontal gyrus, precuneus, left superior temporal gyrus, right precentral gyrus, left anterior cingulate cortex, and right occipital cortex (AlphaSim-corrected $p < .05$) than HCs (Figure 2b and Table 2). For the heartbeats > pure tones contrast, patients with GAD showed greater activity in the left insula, right anterior insula, and right superior frontal gyrus (AlphaSim-corrected $p < .05$) than HCs (Figure 2c and Table 2). We further divided left insula into left anterior insula and left posterior insula. After extracting β values from the left anterior and posterior insula, right anterior insula and right superior frontal gyrus during the conditions of heartbeats > pure tones contrast, posthoc comparisons revealed that heartbeats led to greater activity in these brain areas in GAD patients than HCs ($p < .05$; Figure 3).

TABLE 2 Brain regions exhibiting differences in the hemodynamic response between GAD and HC

Side/Location	Peak coordinates x	y	z	t	Cluster numbers
GAD > HC					
Heartbeat>fixation					
Left anterior insula	−33	0	+18	3.86	57
HC > GAD					
Pure tones > fixation					
Right occipital gyrus	+45	−87	+3	5.04	77
Left superior frontal gyrus	−21	+48	+30	4.33	179
Left anterior cingulate cortex	−9	+39	+9	3.79	118
Right precentral gyrus	+48	−18	+60	3.75	93
Left superior temporal gyrus	−39	−39	+12	3.67	58
Precuneus	0	−45	+39	3.1	59
GAD > HC					
Heartbeat > pure tones					
Left posterior insula	−36	−24	+24	3.45	97
Left anterior insula	−35	0	+18	3.4	142
Right superior frontal gyrus	+27	−12	+75	3.33	59
Right anterior insula	+30	−12	+18	3.29	55

Abbreviations: GAD, generalized anxiety disorder; HC, healthy control.

3.3 | Resting-state FC (rsFC)

Resting-state analyses focused on regions showing significantly higher activity in the patients versus HCs in the heartbeats > pure tones contrast (left anterior insula, right anterior insula, left posterior insula, and right superior frontal gyrus).

As the heartbeats > pure tones contrast yielded elevated left anterior insula activity in patients > HCs, we found that elevated activity in the left anterior insula of patients was paralleled by significantly reduced seed-based rsFC of the left anterior insula cluster with bilateral putamen and left medial PFC (Figure 4a; AlphaSim-corrected $p < .05$; Table 3).

As the heartbeats > pure tones contrast yielded greater left posterior insular activity in patients than in HCs, we found that the elevated activity in the left posterior insula of patients was paralleled by significantly reduced seed-based rsFC in the left posterior insula cluster, including the occipital cortex, right medial PFC, and left supplementary motor cortex (SMA; Figure 4b; AlphaSim-corrected $p < .05$; Table 3).

As the heartbeats > pure tones contrast yielded greater right anterior insula activity in patients than in HCs, we found that the elevated activity in the right anterior insula of patients was paralleled by significantly reduced seed-based rsFC in the right anterior insula cluster, including the bilateral precentral gyrus, right lingual gyrus, left SMA, left superior temporal cortex, and brain stem (Figure 4c; AlphaSim-corrected $p < .05$; Table 3).

As the heartbeats > pure tones contrast yielded greater right superior frontal gyrus activity in patients than in HCs, we found that the elevated activity in the right superior frontal gyrus of patients was paralleled by significantly reduced seed-based rsFC in the right superior frontal gyrus cluster, including the left superior parietal lobule, right calcarine cortex, bilateral

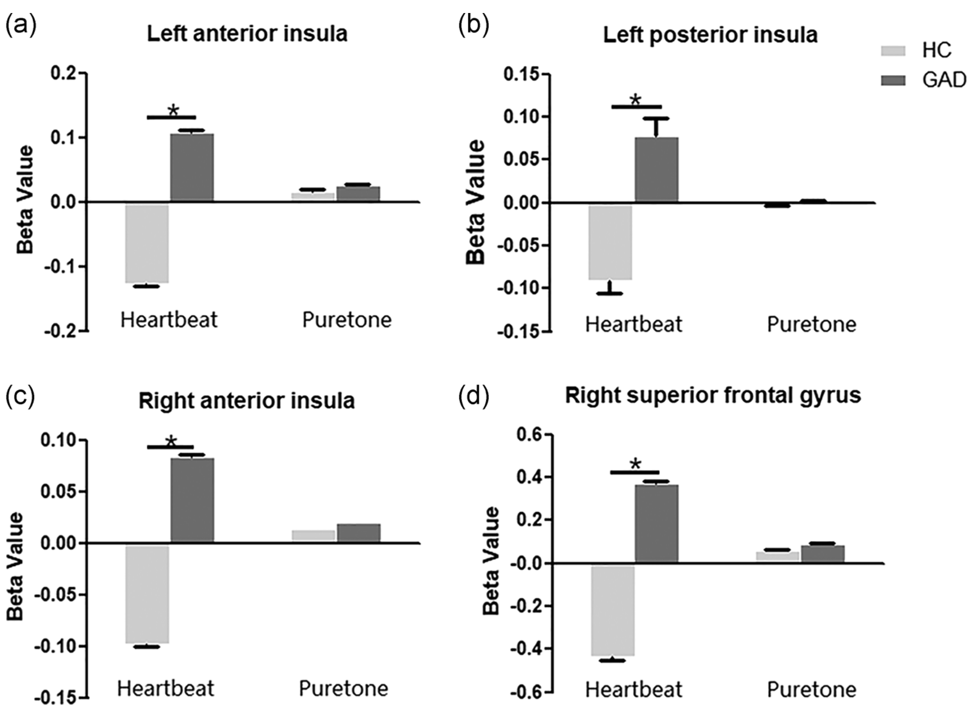

FIGURE 3 Mean betas of left anterior insula (a), left posterior insula (b), right anterior insula (c) and right superior frontal gyrus (d) during the condition of heartbeats > pure tones contrast. Error bars depict the standard error of the mean. *$p < .05$

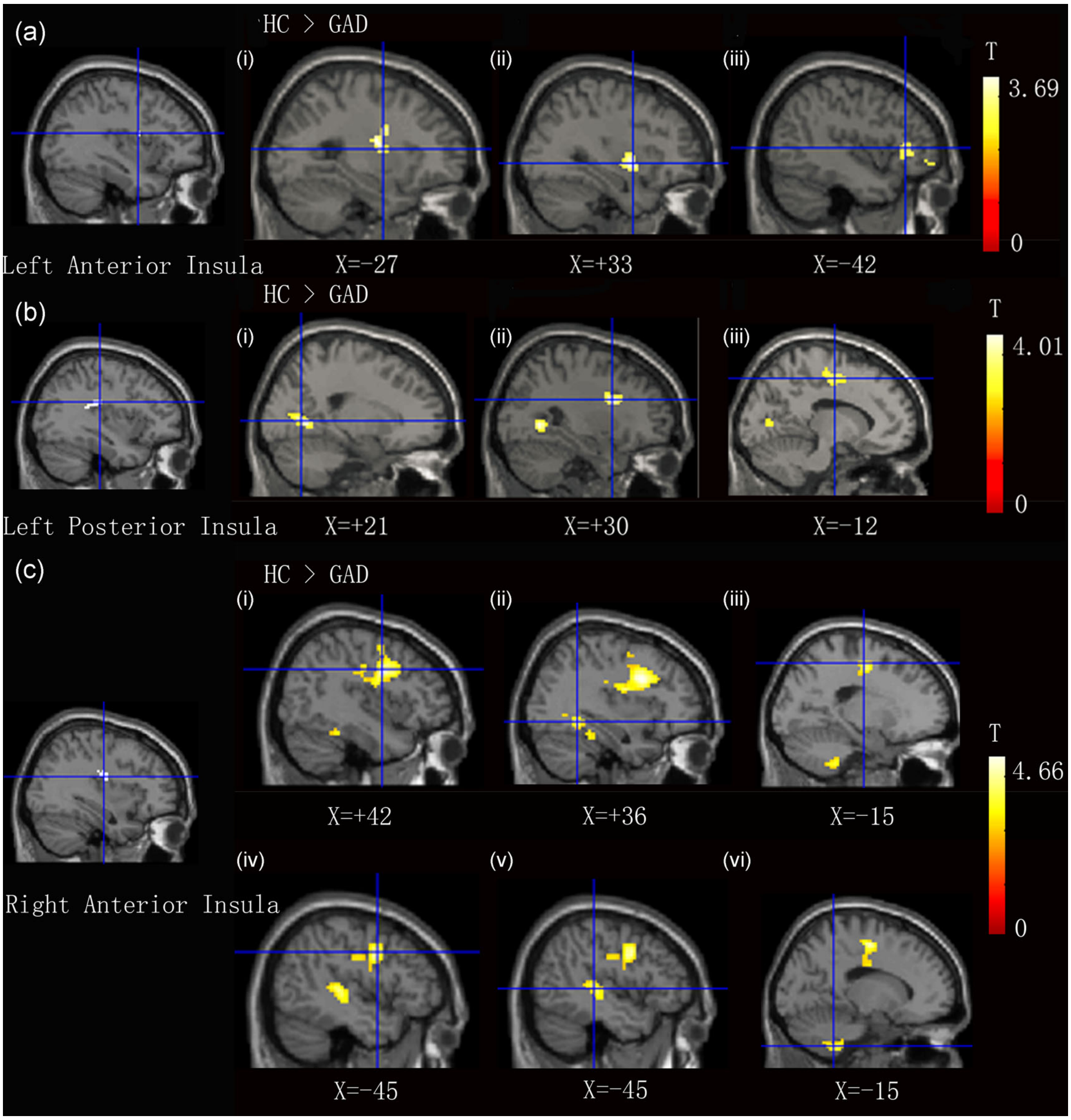

FIGURE 4 (a) Elevated activity in the left anterior insula of patients with GAD in the heartbeats>pure tones contrast was paralleled by significantly reduced seed-based FC between the left anterior insula cluster and the left putamen (i), right putamen (ii), and left medial frontal gyrus (iii); $p < .05$, corrected. (b) Elevated activity in the left posterior insula of patients with GAD in the heartbeats>pure tones contrast was paralleled by significantly reduced seed-based FC between the left posterior insula cluster and occipital cortex (i), right prefrontal cortex (ii), and left supplementary motor area (iii); $p < .05$, corrected. (c) Elevated activity in the right anterior insula of patients with GAD in the heartbeats > pure tones contrast was paralleled by reduced seed-based FC between the right anterior insula cluster and right precentral gyrus (i), right lingual gyrus (ii), left supplementary motor area (iii), left prefrontal cortex (iv), left superior temporal cortex (v), and brain stem (vi); $p < .05$, corrected. FC, functional connectivity; GAD, generalized anxiety disorder; HC, healthy control

occipital pole, right precuneus, right postcentral gyrus, and left precentral gyrus (Table SI1).

3.4 | Correlations between brain activity and anxiety severity

In the heartbeats > pure tones contrast, the blood oxygenation-level-dependent activity in the left anterior insula of patients with GAD exhibited a positive correlation with BPQ-awareness subscale scores (Figure 5). Within the GAD group, the HAMA somatic subscale score was negatively associated with FC between the left anterior insula and left medial prefrontal gyrus (Figure 6).

3.5 | Correlations between task-evoked activity and rsFC

Left anterior insular activity identified in the heartbeats > pure tones contrast was negatively correlated with FC between the left anterior insula and right putamen during resting state in patients with GAD (Figure 7).

TABLE 3 Brain regions exhibiting insula resting–functional connectivity in GAD compared with HC

	Peak coordinates				
Side/Location	x	y	z	t	Cluster numbers
ROI, L anterior insula					
HC > GAD	−27	−9	+18	3.69	82
Left putamen					
Right putamen	+33	+3	−3	3.51	60
Left medial prefrontal cortex	−42	+30	+6	3.15	68
ROI, L posterior insula					
HC > GAD	+21	−66	+3	4.01	329
Occipital cortex					
Right medial prefrontal cortex	+30	+9	+24	3.95	109
Left supplementary motor cortex	−12	−9	+51	3.38	70
ROI, R anterior insula					
HC > GAD	+42	0	+33	4.66	354
Right precentral gyrus					
Right lingual gyrus	+36	−51	−9	4.39	132
Left supplementary motor cortex	−15	−12	+54	4.16	80
Left precentral gyrus	−45	+3	+36	4.10	287
Left superior temporal cortex	−45	−30	+3	3.92	154
Brain stem	−15	−48	−48	3.59	66

Abbreviations: GAD, generalized anxiety disorder; HC, healthy control.

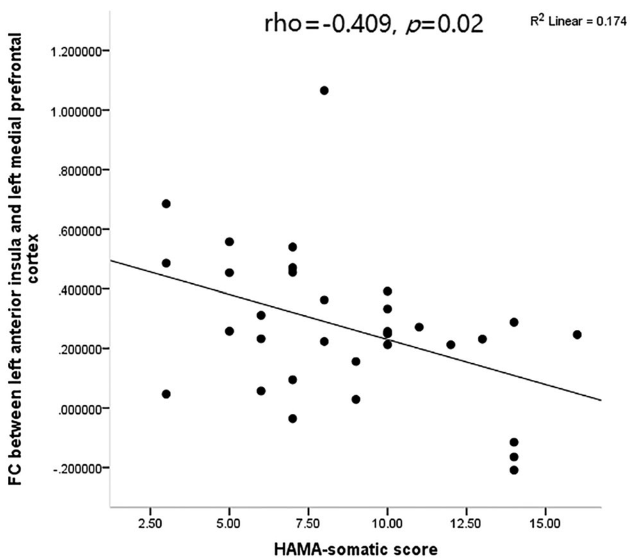

FIGURE 6 Resting-state FC between the left anterior insula and left medial prefrontal gyrus was negatively correlated with somatic anxiety severity. FC, functional connectivity; HAMA, Hamilton Anxiety Scale

4 | DISCUSSION

We investigated the psychological and neural substrates of somatic interoceptive symptoms in a sample of patients with first-episode, drug-free GAD. Psychologically, patients with GAD exhibited increased interoceptive awareness, as tested for by the BPQ. Neurally, they showed increased task-evoked activity in the anterior insula during an interoceptive awareness task. This was accompanied by abnormal rsFC from the same anterior insula regions to other regions that are implicated in cognitive processing, for example, FC between anterior insula and medial PFC in decision making. Neural abnormalities in the left anterior insula during both task and rest correlated with clinical measures. Finally, task-evoked activity in the left anterior insula was negatively correlated with FC between

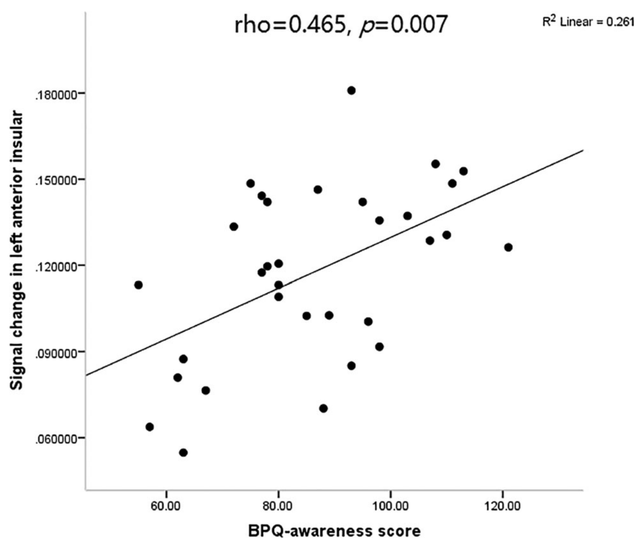

FIGURE 5 Left anterior insula activation in the heartbeats > pure tones contrast was positively correlated with the Body Perception Questionnaire (BPQ)-awareness score

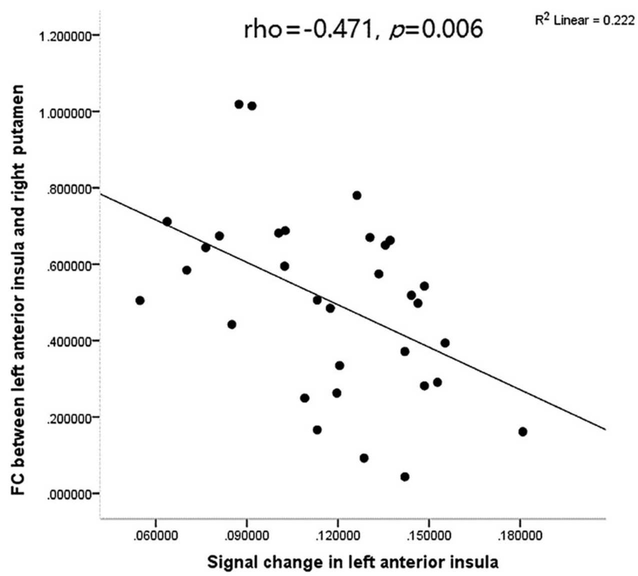

FIGURE 7 Task-evoked activity in left anterior insula was negatively correlated with resting-state functional connectivity (FC) between the left anterior insula and right putamen

the left anterior insula and right putamen during rest. Taken together, the present study demonstrated abnormal psychological activity (interoceptive awareness) and neural activity (resting and task-related) in the left anterior insula, of somatic interoceptive symptoms in first-episode, drug-free GAD, confirming our initial hypotheses.

Patients with GAD showed significantly higher BPQ scores than healthy controls (HCs) in the present study. Higher BPQ scores reflected hypersensitive interoception in GAD, which is consistent with previous findings showing abnormal bodily interoception in patients with GAD (Cui et al., 2016; Mallorqui-Bague et al., 2016; Rossignol, Philippot, & Vogele, 2016). Our results broadened these previous findings by confirming that, in four body perception domains (awareness, stress response, ANSR, and stress styles), values are elevated in patients with GAD.

The posterior insula plays a role in processing the physical features of interoceptive information, whereas the anterior insula integrates these interoceptive physical features with cognitive information (Babo-Rebelo, Wolpert, Adam, Hasboun, & Tallon-Baudry, 2016; Kuehn, Mueller, Lohmann, & Schuetz-Bosbach, 2016). Information from inner organs, such as heart rate, is initially transferred to the posterior insula for basic mapping; afterward, the information is transferred to the anterior insula for more advanced conscious perception (Craig, 2002; Kuehn et al., 2016). The present results demonstrated abnormal task-evoked activity in both the anterior and posterior insula in patients with GAD. Moreover, insular activity is related to interoceptive awareness in the anterior insula, consistent with our a priori hypothesis; activity in the left anterior insula had a positive correlation with BPQ-awareness score during the interoceptive awareness task, whereas FC between the left anterior insula and left medial PFC had a negative correlation with HAMA-somatic score during rest. In this regard, we have expanded upon previous findings of insular changes in patients with GAD (Buff et al., 2016; Karim et al., 2017; Moon & Jeong, 2017; Qiao et al., 2017; Santos, Carvalho, van Ameringen, Nardi, & Freire, 2018) because we showed elevated anterior insular activity during both interoceptive awareness and rest, with both being related to psychological/behavioral measures.

Resting-state fMRI studies have indicated extensive FC between the insula and other brain areas (Uddin, Nomi, Hebert-Seropian, Ghaziri, & Boucher, 2017), providing indirect evidence for the role of FC in GAD. The anterior insula has connections to the frontal, anterior cingulate, parietal, and limbic areas, and these are involved in cognitive control and affective processes (Nomi et al., 2016; Steward et al., 2016). And, the posterior insula has connections with the temporal and posterior cingulate areas involved in sensorimotor processes (Nomi et al., 2016). In addition to altered task-evoked insular activity in patients with GAD, we found abnormal rsFC from the anterior and posterior insula to various regions, notably the medial PFC, putamen, occipital cortex, precentral gyrus, temporal cortex, and SMA. This may suggest that cognitive control, affective processes and sensorimotor processing are disrupted in GAD. Furthermore, the anterior insula plays roles in processing cognitive decisions by connecting with the medial PFC (Weller, Levin, Shiv, & Bechara, 2009), which may contribute to the psychopathology of GAD. Our observation that decreased FC between the left anterior insula and left medial PFC is correlated with somatic anxiety symptoms in GAD supports this assumption.

5 | LIMITATIONS

Several limitations should be noted. First, to assess interoceptive awareness at the behavioral level, we chose BPQ scales instead of a heartbeat counting task, the interoceptive accuracy scores from which might be confounded due to some potential weaknesses (Zamariola, Maurage, Luminet, & Corneille, 2018). BPQ is a self-administered scale that has not been normed. However, at least 30 peer-review papers have used the BPQ in their research (see Table S1), and a recent study investigating the psychometric properties of the Body Awareness and Autonomic Reactivity subscales in the BPQ showed good reliability and validity (Cabrera et al., 2018). We used the BPQ in our previous study (Cui et al., 2016) and showed good reliability in a Chinese sample. In the Supporting Information Materials, we have provided the methods and results of heartbeat accuracy scores in this study for readers' review, as shown in Table SIII. The second limitation of the present study was the region-of-interest approach that we adopted, which was hypothesis-driven and may, therefore, have disregarded results from other brain areas. However, the insula-of-interest approach makes it easier for other researchers to replicate our results. Finally, one considerable merit of our study was that it involved first-episode patients with GAD who were not taking any psychotropic medications. To investigate dynamic changes in the neural activity of insula and related FC networks, further prospective studies should be carried out before and after treatment.

6 | CONCLUSIONS

In the present study, we demonstrated the psychological and neural substrates of somatic interoceptive symptoms in a sample of patients with first-episode, drug-naïve GAD. Psychologically, GAD patients exhibited higher body perception scores, which also correlated with anxiety symptoms. Task-evoked activity during interoceptive awareness in the left anterior insula was abnormally elevated in GAD, accompanied by abnormal rsFC between that region and others implicated in cognitive and affective processing. Importantly, abnormalities in both resting state and task-evoked activity correlated with clinical measures. Together, our findings demonstrate the psychological and neural substrates of abnormal interoception and somatic vegetative symptoms in patients with GAD. In addition to pathophysiological understanding, this carries major implications for the development of future diagnostic and therapeutic markers in GAD.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

DATA AVAILABILITY STATEMENT

Data will be made available in accordance with the NIMH data-sharing agreement.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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