Biological Psychology 174 (2022) 108409

Contents lists available at ScienceDirect

Biological Psychology

journal homepage: www.elsevier.com/locate/biopsycho

Altered local gyrification index and corresponding resting-state functional connectivity in individuals with high test anxiety

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ARTICLE INFO

Keywords:
Executive control network
Magnetic resonance imaging
Sensorimotor network
Visual network

ABSTRACT

Previous studies have reported that test anxiety is closely related to unreasonable cognitive patterns and maladaptive emotional responses. However, its underlying brain structural and functional basis has not been thoroughly studied. This study aimed to evaluate the potential difference in local gyration index (LGI) and corresponding resting-state functional connectivity (RSFC) in individuals with high test anxiety (HTA) compared with low test anxiety (LTA). Twenty-six individuals with HTA and 28 individuals with LTA underwent T1-weighted structural and resting-state functional magnetic resonance imaging scans. Using FreeSurfer software, we contrasted the LGI between the HTA and LTA groups using a surface-based general linear model to map group contrasts on a vertex-by-vertex basis. By selecting the cortical regions with significant differences in the LGI analysis as the regions of interest, the seed-based RSFC analysis was further carried out using the Resting-State fMRI Data Analysis Toolkit to examine the differences in the functional connectivity of these cortical regions with the whole brain between the two groups. The results showed that the LGI in several cortical regions of the executive control network (ECN) and the right lateral occipital gyrus was lower in the HTA group than in the LTA group. Furthermore, compared with the LTA group, the HTA group exhibited abnormal RSFC within the ECN, between the ECN and the visual network, and between the ECN and the sensorimotor network. Our findings might provide preliminary evidence for brain morphology and functional alterations in individuals with HTA and contribute to a better understanding of the pathophysiology of TA.

1. Introduction

Most individuals feel anxious and nervous when facing important exams. Individuals that regard exams as a threat, experience intense worries and emotions, and undergo corresponding physiological and behavioral reactions are said to experience test anxiety (Liebert & Morris, 1967). As Hong (1998) suggested, test anxiety can be divided into state test anxiety, which refers to the transitory, anxious affect state provoked by a specific evaluative situation, and trait test anxiety, which refers to the tendency to be anxious in any evaluative situation. Trait test anxiety not only is more stable than state test anxiety (Hong, 1998) but also has greater stability in predicting negative consequences. For example, trait test anxiety can affect the mental health of adolescents (Zeidner, 1998) and predict subsequent depression and anxiety levels in individuals (Akinsola & Nwajei, 2013; Beer, 1991). Therefore, researchers are more interested in inter-individual differences in test anxiety (rather than intraindividual variability over time or occasions) and its causes and interventions, that is, they have focused more on trait test anxiety than on state test anxiety (e.g., Shen et al., 2018; Song et al., 2021, 2022; Von Der Embse et al., 2013). In addition, trait test anxiety implies individual differences, and researchers usually tend to refer to it simply as "individuals with high test anxiety/low test anxiety" (HTA/LTA) rather than "individuals with high trait test anxiety/low trait test anxiety." For these reasons, this study also focused on the differences between individuals with high trait test anxiety and low trait test anxiety and followed the tradition of using the term “individuals with high test

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https://doi.org/10.1016/j.biopsycho.2022.108409
Received 14 May 2022; Received in revised form 2 August 2022; Accepted 15 August 2022
Available online 18 August 2022

anxiety/low test anxiety" (i.e., HTA/LTA, respectively).

Several instruments are used to measure test anxiety, such as the Test Anxiety Questionnaire (TAQ, Mandler & Sarason, 1952), the Test Anxiety Scale (TAS, Sarason, 1977), the Worry-Emotionality Questionnaire (Morris & Liebert, 1970), the Inventory of Test Anxiety (Osterhouse, 1972), and the Test Anxiety Inventory (Spielberger, 1980). Among these, the TAQ was the first instrument to measure test anxiety, and the other scales were developed on the basis of the TAQ, which used a less convenient graphic rating method. Most studies used the TAS to screen participants with HTA/LTA (e.g., Hu et al., 2022; Huynh et al., 2022; Liu et al., 2021; Roshanisefat et al., 2021), given that the TAS was considered the most popular instrument to measure test anxiety (Aydin et al., 2020; Tryon, 1980) and had a clear and widely used cutoff (Newman, 1996), whereas the high/low grouping criteria of other scales were influenced by the sample size and were somewhat arbitrary.

Previous studies reported that the most significant characteristics of test anxiety were emotionality and worry (Liebert & Morris, 1967; Spielberger, 1980). Emotionality refers to the tension that an individual may experience and the corresponding physiological reactions, including a rapid heartbeat, a cold sweat, shortness of breath, and trembling; whereas worry refers to the thoughts before, during, and after the test, such as worrying about failing the test, the performance of the other students, and the ranking of the test results (Putwain & Pescod, 2018). Emotionality and worry further lead to a series of unreasonable cognitive patterns and maladaptive emotional responses, such as defects in attention control (Keogh & French, 2001; Lawson, 2006; Putwain & Daly, 2014; Zhang & Zhou, 2015; W. Zhang et al., 2019), visual working memory filtering (Song et al., 2021), stress (Conley & Lehman, 2012; Conneely & Hughes, 2010), and emotional responses (Ringeisen & Buchwald, 2010). However, the neural mechanism of these abnormal cognitive and emotional processes is not yet clear.

Since the advent of magnetic resonance imaging (MRI), many morphometric analysis methods have been developed to identify the macro-structural changes in the human brain, which can be roughly divided into voxel-based morphometry (VBM; Ashburner & Friston, 2000; Bora et al., 2012; Schienle et al., 2011; Strawn et al., 2013) and surface-based morphometry (SBM; Palaniyappan & Liddle, 2012). As the most commonly-used VBM indicator (i.e., gray matter volume) includes information on the cortical thickness, surface area, and cortical folding, researchers contend that greater accuracy exists in the direct investigation of SBM indicators (i.e., thickness, surface area, and cortical folding) in the study of specific structural alterations in populations with psychiatric disorders (Miskovich et al., 2016).

Cortical folding, which is measured by the local gyrification index (LGI; Schaer et al., 2008; Zilles et al., 1988), not only occurs during the fetal and early postnatal phases (Zilles et al., 2013) but also throughout adolescence and adulthood (Hogstrom et al., 2013; Klein et al., 2014; White et al., 2010). It is significantly affected by genes but also determined by nongenetic factors (Bartley et al., 1997; White et al., 2002). Thus, it can reflect the changes in brain structure that occur during development. Test anxiety is affected by individual biological and social factors (e.g., family, school, and test environment) (Gao & Liu, 2021), and hence cortical folding can better reflect the changes in brain structure caused by test anxiety.

Previous empirical studies using the LGI identified abnormalities in the brain structure of adult clinical samples of anxiety and depression. For example, compared with healthy participants, patients with major depressive disorder exhibited a decreased LGI in the bilateral middle posterior cingulate gyrus, insula, orbitofrontal cortex, left anterior cingulate cortex, right temporal cortex, and left angular gyrus, whereas individuals with generalized anxiety disorder exhibited an increased LGI in the right fusiform gyrus, inferior temporal gyrus, superior parietal gyrus, and left superior frontal gyrus (Long et al., 2020; Zhang et al., 2009). In addition, not only were changes in LGI found in people clinically diagnosed with depression or anxiety disorders, but previous studies also demonstrated that trait anxiety was negatively correlated with the LGI of the left precuneus (Miskovich et al., 2016). Therefore, the LGI can effectively and sensitively reflect the changes in the brain structure of those who are emotionally disturbed (though not meeting the clinical diagnostic criteria).

In general, this study first used TAS to select individuals with HTA and LTA, then used a surface-based approach to examine the differences in brain structure between the two groups using LGI as an indicator, and further used the brain regions with group-related differences in LGI as the regions of interest to examine the differences in RSFC between the two groups. According to the attentional control theory (Eysenck et al., 2007) and empirical studies (see W. Zhang et al., 2019), one of the most typical cognitive features of test anxiety was attentional control deficit, which was also closely related to other abnormal cognitive and emotional processes (Spada et al., 2010; Stefanopoulou et al., 2014). Therefore, we hypothesized that the brain structural and RSFC abnormalities of individuals with HTA mainly occurred in brain regions (e.g., prefrontal cortex; Sylvester et al., 2012) and RSFC (e.g., anterior cingulate cortex and prefrontal cortex; Basten et al., 2011, 2012) associated with attentional control. However, since previous studies had not examined the differences in brain structure and RSFC between the two groups, we did not make specific hypotheses for the time being.

2. Material and methods

2.1. Participant screening

This study was approved by the Ethical Evaluation of Research Projects at the Department of Psychology in the School for Social and Behavioral Sciences at Nanjing University. All participants provided written informed consent upon arrival at the laboratory. All procedures involving human participants were performed in accordance with the ethical standards of the institutional or national research committee and the 1964 Declaration of Helsinki as well as its later amendments or comparable ethical standards.

The desired sample size was based on G*Power analysis. We set the effect size d as 0.8, α as 0.05, and power as 0.8 for the difference between the two independent means (two groups), and the G*Power produced a recommended sample size of 26 participants per group. We recruited college students (including undergraduate and graduate students) aged 18–25 years through posters or online advertisements at universities. Then, 172 interested college students completed the online survey, which included TAS (Sarason, 1977), the Beck Depression Inventory (BDI; Beck et al., 1961), and some other questions, which formed our inclusion criteria (see the details below).

According to the TAS cutoff (Newman, 1996), those with scores higher than 20 were classified into the HTA group and those with scores lower than 12 were classified into the LTA group. Then, all potential participants who met the HTA or LTA requirements further needed to meet the following inclusion criteria according to their online self-reporting: right-handed; no history of diagnosed psychiatric disorders or neurological problems; no use of mood-altering substances in the last 6 months; no metals inside the body; no history of physical or mental diseases affecting MRI, such as claustrophobia, cervical spondylosis, or tympanitis; no history of self-reported depression treatment; and no severe depression (i.e., BDI scores < 21).

Then, we invited participants who met the aforementioned inclusion criteria (including TAS and BDI scores requirements) and volunteered to participate in the study to our lab in their free time. Upon arrival at the lab, the potential participants first signed an informed consent form and then completed the Chinese version of the Mini International Neuropsychiatric Interview (MINI), based on the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV), and the International Statistical Classification of Mental Disorders (ICD-10) (Si et al., 2009), to exclude 16 kinds of Axis I psychiatric disorders and antisocial personality disorder. Finally, if the interview confirmed neither Axis I psychiatric disorders nor antisocial personality disorder, they

participated in MRI data collection and received appropriate payment before leaving the lab.

Consequently, 27 individuals with HTA and 29 individuals with LTA formed the sample for this study. One participant in each group was excluded from data analysis due to excessive movement (mean FD_Jenkinson > M + 2 SD) during resting-state scanning. Significant differences were found in TAS scores between the HTA ($n = 26$; 21 female) and LTA ($n = 28$; 12 female) groups (25.62 ± 3.57 vs 7.82 ± 2.45, t [52] = 21.50, $p < .001$). However, significant differences were also found in age (21.38 ± 2.08 vs 22.68 ± 2.06, t [52] = −2.30, $p = .03$) and sex ratio (χ^2 [1] = 8.15, $p = .004$).

2.2. Materials

2.2.1. Test anxiety scale

The TAS (Sarason, 1977), which was translated into Chinese (Wang, 2001), was used to select individuals with HTA and LTA. The TAS contained 37 items rated as true or false. The higher the total score, the higher the number of test anxiety symptoms experienced in the evaluation situation. Although the reliability of the initial revised Chinese version was only within the acceptable range (the test-retest reliability of the scale was.61, and the homogeneity coefficient was.64; Wang, 2001), recent studies showed that the Chinese version of TAS had good reliability, with Cronbach's alpha coefficients above.80 (e.g., Liu et al., 2021; Xu et al., 2020; Wei et al., 2022). In this study, Cronbach's alpha coefficient and the composite reliability (95% confidence interval), which were obtained from the results of the confirmatory factor model (see Raykov, 1998; Raykov & Shrout, 2002; Ye & Wen, 2011), of the scale were .78 and .77 [.72 .82], respectively. Furthermore, we also used another scale measuring test anxiety (i.e., TAI; Spielberger, 1980) to calculate the external validity of TAS. The results showed a higher correlation between the scores of the two scales ($r = .77$, $p < .001$), indicating that the TAS also had good validity. Furthermore, it is also commonly used to screen for test anxiety because it has a fixed cutoff, as proposed by Newman (1996), and has the advantage of not being subject to sample size and not being arbitrary compared with other scales (e.g., TAI). In summary, it was used in this study to screen individuals with HTA and LTA.

2.2.2. Beck depression inventory

The BDI (Beck et al., 1961), translated into Chinese (Zhang et al., 1990), was used to exclude participants with severe depression affect. The scale included 21 items, rated on a 4-point scale from 0 (no) to 3 (extremely heavy) to indicate the extent to which the items reflected participants' affect. After revising the Chinese version, Cronbach's alpha coefficient was.85 and the test-retest reliability was.73 after 1 week (Zhang et al., 1990). In this study, Cronbach's alpha coefficient of this scale was.92. According to the cutoff, those with scores higher than or equal to 21 were excluded from this study.

2.2.3. Mini International Neuropsychiatric Interview

The MINI (Sheehan et al., 1997), translated into Chinese (Si et al., 2009), was used to exclude participants with 16 kinds of Axis I mental disorders (including major depressive episode, major depressive episode with melancholic features [optional], dysthymia, suicidality, [Hypo] manic episode, panic disorder, agoraphobia, social anxiety disorder, obsessive–compulsive disorder, posttraumatic stress disorder, alcohol abuse and dependence, nonalcohol psychoactive substance use disorders, psychotic disorder, anorexia nervosa, bulimia nervosa, and generalized anxiety disorder) and antisocial personality disorder (optional), according to DSM-IV and the ICD-10. After the revision of the Chinese version, the interrater and test-retest kappa values were .94 and .97–1.00, respectively. In addition, using the structured clinical interview for the Diagnostic and Statistical Manual of Mental Disorders, Third version, Revised (SCID) (Spitzer et al., 1992) as the gold standard, the validity of the Chinese version MINI ranged from.76 to .88. The MINI is widely used in the participant screening phase of the research (e.g., Hou et al., 2019) and clinical assessment (e.g., Pettersson et al., 2018) due to its high reliability and significantly shorter time spent compared with other structured interviews (e.g., SCID).

2.3. Data acquisition

MRI data were collected using a Siemens 3.0 T magnetic resonance scanner (Siemens Medical, Germany). The heads of the participants were fixed with MRI-compatible foam to reduce head movement. Before scanning, we asked all participants to change into the provided clothing to ensure that no metal objects affected the participants' safety and the image quality. In addition, we requested that all metal jewelry and dentures worn by participants be removed. During scanning, all participants were asked to open their eyes and look at the fixation on the screen, to keep their head still, and not to think about anything in particular.

High-resolution T1-weighted brain structures were acquired using a magnetization-prepared rapid gradient echo (MPRAGE) sequence (TR = 2600 ms; TE = 3.02 ms; FA = 8°; 256 × 256 matrix; 176 slices; 1.00-mm slice thickness; voxel size = 1 × 1 × 1 mm^3), whereas T2*-weighted images were recorded using an echo-planar imaging (EPI) sequence (TR = 2000 ms; TE = 30 ms; flip angle = 90°; field of view (FOV) = 240 mm × 240 mm; matrix size = 64 × 64; 33 interleaved 3-mm-thick slices; in-plane resolution = 3.4 mm × 3.4 mm; interslice skip = 1 mm; volumes = 240).

2.4. Data analysis

2.4.1. LGI analysis

Each structural scan was processed using the FreeSurfer 6.0 image analysis suite (http://surfer.nmr.mgh.harvard.edu/) with the following steps. First, the data from all participants were processed using the fully automated FreeSurfer "recon-all" standard procedure (Dale et al., 1999), which included nonuniform intensity correction, skull stripping, Talairach transformations, normalization and atlas registration, subcortical segmentation, surface reconstruction, cortical atlas registration and segmentation, and other processes. Second, using the method proposed by Schaer et al. (2008), the LGI was calculated by measuring the ratio of the local surface area to the outer hull layer that tightly wrapped the pial surface. This method was an improvement of the two-dimensional method proposed by Zilles et al. (1988). It not only considered the three-dimensional attribute of cortical folding, but it also could be automatically calculated to reduce subjectivity. Finally, each vertex-wise LGI value was mapped using a common spherical coordinate system (fsaverage) and smoothed with a 5-mm Gaussian kernel.

2.4.2. RSFC analysis

All the functional MRI (fMRI) data were preprocessed using a toolbox for Data Processing Assistant for Resting-State fMRI (DPARSFA; http://www.restfmri.net/forum/DPARSF; Yan & Zang, 2010) with the following steps. First, the original DICOM data were converted into the NIFTI data format, and the first 10 volumes were discarded. Second, slice timing was performed to correct within-scan acquisition differences between slices. Third, all the remaining images were registered to the first volume to correct the head motion and exclude participants with excessive movement (mean FD_Jenkinson > M + 2 SD). After excluding these participants, no significant difference was found in the head movement between the two groups (mean FD_Jenkinson: 0.06 ± 0.02 vs 0.06 ± 0.03, t [52] = –0.36, $p = .72$). Fourth, the fMRI was co-registered to the T1 structural image using the diffeomorphic anatomic registration through an exponentiated lie algebra algorithm (DARTEL). Fifth, the covariates were removed, which included Friston-24 head motion parameters, white matter, and cerebrospinal fluid signals. Sixth, spatial standardization was performed using the DARTEL method to register the image to the standard Montreal

Neurological Institute (MNI) space. Seventh, Gaussian smoothing was performed with a 4-mm Gaussian kernel. Finally, band-pass filtering was performed with a filtering range of 0.01–0.10 Hz.

2.4.3. Statistical analysis

For LGI analysis, a general linear model (GLM) with age and sex as covariates was adapted to estimate group differences in the LGI value at each vertex, where the Query Design Estimate Contrast (Qdec) application embedded in the FreeSurfer program was used to generate the contrasts, due to the unmatched age and sex ratio between the two groups. Thereafter, the significant regions were saved as label files, and the "ch2″ in SPM12 (https://www.fil.ion.ucl.ac.uk/spm/software/spm12/) was used as a bridge to transform these label files from the fsaverage standard space into the SPM standard space as ROIs for RSFC analysis. The specific steps were as follows. First, the "ch2″ was processed using the fully automated FreeSurfer "recon-all" standard procedure. Second, the label2label function was used to convert the significant label files from the fsaverage standard space into the "ch2″ individual space. Third, the "ch2″ of the fsaverage standard space was registered to the SPM standard space using the tkregister2 function, and the mapping parameters were obtained. Finally, the mri_label2vol function was used to convert the label generated in the second step into the ROI in the NIFTI format based on the mapping parameters generated in the third step.

For RSFC analysis, the Resting-State fMRI Data Analysis Toolkit (REST) (http://restfmri.net/forum/REST_V1.8; Song et al., 2011) was used to calculate the voxel-wise functional connectivity between the aforementioned ROIs and the whole brain, and then a GLM in SPM12 with age, sex, and head movement (i.e., mean FD_Jenkinson) as covariates was adapted to estimate group differences in the RSFC. Age and sex were used as covariates because of the mismatch between the two groups, whereas the head movement parameter was used as a covariate because the head movement might affect the comparison of the two groups of RSFC (see Yan et al., 2013).

The RSFC values did not conform to the normal distribution, and hence we carried out Fisher *Z*-transformation and used the *Z*-values for RSFC statistical analysis. In addition, the LGI and RSFC results were corrected for multiple comparisons with a cluster-wise level of $p < .05$, and a vertex/voxel-wise level of $p < .005$ using the Monte Carlo simulation and the familywise error (FWE), respectively.

3. Results

3.1. LGI analysis

As shown in Table 1 and Fig. 1, the differences in the LGI between the two groups were mainly located in the bilateral temporal and frontal cortices. Specifically, the LGI values for the bilateral rostral middle frontal gyrus (RMFG), left caudal middle frontal gyrus (CMFG), left superior temporal gyrus (STG), left inferior temporal gyrus (ITG), right superior frontal gyrus (SFG), and right lateral occipital gyrus (LOG) were lower in the HTA group than in the LTA group.

3.2. RSFC analysis

As shown in Table 2, the RSFC values in the left RMFG–bilateral superior parietal gyrus (Fig. 2**a**), left RMFG–bilateral cerebellum (Fig. 2**b**), left RMFG–left postcentral gyrus/paracentral lobule (Fig. 2**c**), left ITG–bilateral postcentral gyrus (Fig. 3**a**), left ITG–right lingual gyrus (Fig. 3**b**), left ITG–right inferior occipital gyrus (Fig. 3**c**), left ITG–left precentral gyrus (Fig. 3**d**), left ITG–right cerebellum (Fig. 3**e**), left CMFG–left orbital ITG (Fig. 4**a**), left STG–left triangle inferior frontal gyrus (Fig. 4**b**), and right LOG–right triangle inferior frontal gyrus (Fig. 4**c**) were lower in the HTA group than in the LTA group, while the RSFC value for the right RMFG–bilateral thalamus (Fig. 4**d**) was higher in the HTA group than those in the LTA group.

4. Discussion

Although previous studies have shown that individuals with HTA account for up to 15–22% of the student population (Putwain & Daly, 2014; Huang & Zhou, 2019), the brain structural and functional bases of TA remain largely unknown. This study was the first to use high-resolution structural MRI and the LGI as an indicator to investigate abnormal cortical folding in individuals with HTA. The LGI in the executive control network (ECN, which included the bilateral RMFG, left CMFG, left STG, left ITG, and right SFG) and the right LOG were lower in the HTA group than in the LTA group. Furthermore, compared with the LTA group, the HTA group showed abnormalities in the RSFC within the ECN (left RMFG–bilateral superior parietal gyrus, left CMFG–left orbital inferior temporal gyrus, and left STG–left triangle inferior frontal gyrus), between the ECN and the visual network (left ITG–right lingual gyrus, left ITG–right inferior occipital gyrus, and right LOG–right triangle inferior frontal gyrus), and between the ECN and sensorimotor networks (left RMFG–bilateral cerebellum, left RMFG–left postcentral gyrus/paracentral lobule, left ITG–bilateral postcentral gyrus, left ITG–left precentral gyrus, left ITG–right cerebellum, and right RMFG–bilateral thalamus).

The ECN, also called the frontal–parietal network, mainly includes the orbital prefrontal cortex, middle frontal gyrus, SFG, inferior frontal gyrus, superior parietal gyrus, inferior parietal gyrus, ITG, and middle temporal gyrus (Geiger et al., 2016). The ECN is associated with attentional control. For example, individuals with high trait anxiety showed reduced functional connectivity between regions of the cingulo-opercular network and the ECN in the word color Stroop task (Basten et al., 2011). Previous studies reported that individuals with HTA were more vulnerable to test-related information than those with LTA (Gao & Zhou, 2013; Keogh & French, 2001; Lawson, 2006; Liu et al., 2015; Putwain et al., 2011; W. Zhang et al., 2019). The researchers further demonstrated that the damage to the attention control of individuals with HTA individuals was not limited to test-related stimuli, but affected all situations where it was necessary for irrelevant

Table 1
Group differences in the LGI.

Cluster-level sig.	Cluster size (mm^2)	*T*	MNI			Regions	Difference directions
			X	Y	Z		
<.001	3586.62	-6.88	-23.3	53.6	14.5	Left RMFG	LTA> HTA
.008	869.66	-4.11	-53.1	-34.3	-22.5	Left ITG	LTA > HTA
.001	1212.35	-3.63	-42.2	13.3	45.5	Left CMFG	LTA > HTA
.001	1326.20	-3.82	-61.4	-46.7	16.8	Left STG	LTA > HTA
<.001	2940.84	-4.58	43.3	-80.8	-2.7	Right LOG	LTA > HTA
.002	1066.70	-4.19	29.5	41.5	18.6	Right RMFG	LTA > HTA
.006	889.22	-3.27	9.0	8.5	52.4	Right SFG	LTA > HTA

Notes: RMFG = rostral middle frontal gyrus; ITG = inferior temporal gyrus; CMFG = caudal middle frontal gyrus; STG = superior temporal gyrus; LOG = lateral occipital gyrus; SFG = superior frontal gyrus. The vertex-level statistical threshold was set at $p < .005$ and corrected with a cluster-level of $p < .05$ using the Monte Carlo simulation.

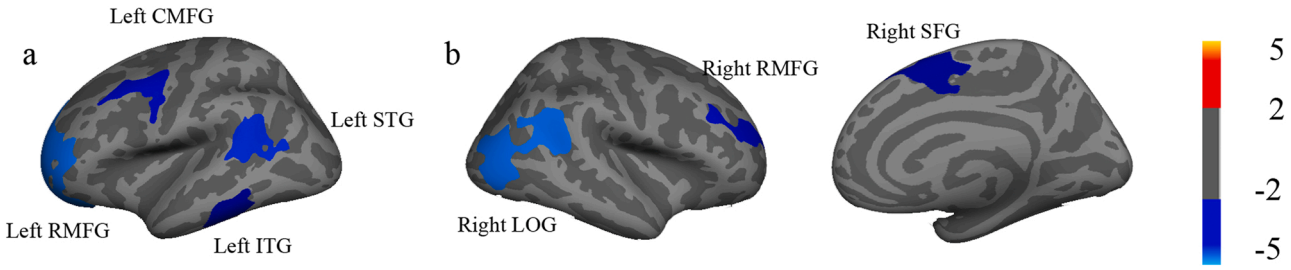

Fig. 1. LGI values in the (a) left rostral middle frontal gyrus (RMFG), caudal middle frontal gyrus (CMFG), superior temporal gyrus (STG), and inferior temporal gyrus (ITG), and (b) right RMFG, superior frontal gyrus (SFG), and lateral occipital gyrus (LOG) were lower in the HTA group than in the LTA group.

Table 2
Group differences in the RSFC.

Cluster-level sig.	Cluster size (voxel)	*T*	MNI			Regions	Difference directions
			X	Y	Z		
ROI: Left RMFG							
<.001	251	4.33	-15	-63	42	Left superior parietal gyrus	LTA > HTA
.01	95	4.32	-21	-39	72	Left postcentral gyrus/ paracentral lobule	LTA > HTA
.002	121	4.09	18	-60	45	Right superior parietal gyrus	LTA > HTA
.006	104	3.90	-39	-75	-3	Left cerebellum	LTA > HTA
.043	73	3.85	36	-72	-21	Right cerebellum	LTA > HTA
ROI: Left ITG							
<.001	1408	5.18	-21	-36	72	Bilateral postcentral gyrus	LTA > HTA
<.001	907	5.60	6	-54	-3	Right lingual gyrus	LTA > HTA
<.001	220	5.19	45	-75	-15	Right inferior occipital gyrus	LTA > HTA
.007	101	6.32	-33	0	63	Left precentral gyrus	LTA > HTA
.043	73	4.72	33	-45	-54	Right cerebellum	LTA > HTA
ROI: Left CMFG							
.001	138	4.37	-51	42	-3	Left orbital inferior temporal gyrus	LTA > HTA
ROI: Left STG							
.017	89	4.4	-54	36	6	Left triangle inferior frontal gyrus	LTA > HTA
ROI: Right LOG							
.043	72	4.95	57	24	18	Right triangle inferior frontal gyrus	LTA > HTA
ROI: Right RMFG							
.028	78	4.01	12	-15	3	Bilateral thalamus	HTA > LTA
ROI: Right SFG							
None							

Notes: RMFG = rostral middle frontal gyrus; ITG = inferior temporal gyrus; CMFG = caudal middle frontal gyrus; STG = superior temporal gyrus; LOG = lateral occipital gyrus; SFG = superior frontal gyrus. The voxel-level statistical threshold was set at $p < .005$ and corrected with a cluster-level of $p < .05$ at the family-wise error (FWE).

information to be ignored (Gao & Zhou, 2013; Liu et al., 2015; W. Zhang et al., 2019). Furthermore, Wei et al. (2020) reported a significant positive correlation between the TAS score and the electroencephalographic theta/beta power ratio, which was associated with attentional control. Therefore, the present study found that the decrease in the LGI in the ECN of individuals with HTA echoed the earlier findings on attention control defects in individuals with HTA and provided a neural structural basis for them.

The LOG is an important visual area (Lee et al., 2000). The present study found that the LGI value for the LOG was lower in the HTA group than in the LTA group. This might reflect the low structural complexity of individuals with HTA in this area, which was similar to previous results on the gray matter volume of individuals with other anxiety disorders (Frick et al., 2014; Wang et al., 2018). During brain development, synaptic pruning and myelination may reduce the gray matter volume in the brain and improve the efficiency of corresponding psychological processes (Kanai & Rees, 2011; Konrad et al., 2013). The increase in the gray matter volume (Frick et al., 2014; Wang et al., 2018) and the decrease in the LGI in highly anxious individuals may reflect the decrease in information transmission efficiency caused by the abnormal development of the visual cortex. Previous studies using electroencephalogram technology indicated that compared with individuals with LTA, individuals with HTA exhibited greater amplitudes of P1 and P2 for test-related threatening words, indicating that the inhibitory deficit observed in individuals with HTA occurred as early as in the perceptual stage (W. Zhang et al., 2019). We used MRI technology to further provide the neural structural basis for this study. For individuals with HTA, the information transmission efficiency in the visual cortex was reduced. Therefore, it was necessary to recruit more of the visual cortex to process relevant test-related information than that for individuals with LTA, which was reflected in the increase in early components (P1 and P2).

We further analyzed the seed-based RSFC differences between the two groups to explore the abnormal functional connectivity caused by abnormal cortical folding. The results showed differences in the RSFC within the ECN, between the ECN and the visual network, and between the ECN and the sensorimotor network between the two groups. Sylvester et al. (2012) proposed that anxiety disorder and high trait anxiety were related to a particular pattern of functional network dysfunction, that is, increased functioning of cinguloopercular and ventral attention networks as well as decreased functioning of the ECN and default mode networks. In other words, in anxious individuals, the alterations in networks supporting emotion processing and in those supporting higher cognition control were observed. The results of this study revealed reduced functional connectivity in individuals with HTA within the ECN. This might reflect the low efficiency of information exchange within the ECN, resulting in an abnormal top-down control process, similar to that found in earlier studies on patients with social anxiety disorders (Geiger et al., 2016) and eating disorders (Chen et al., 2021).

We also observed that individuals with HTA exhibited an altered RSFC between the ECN and the visual network, and between the ECN and the sensorimotor network. This might reflect the long-term impact of test anxiety on visual and sensorimotor processes, that is, the

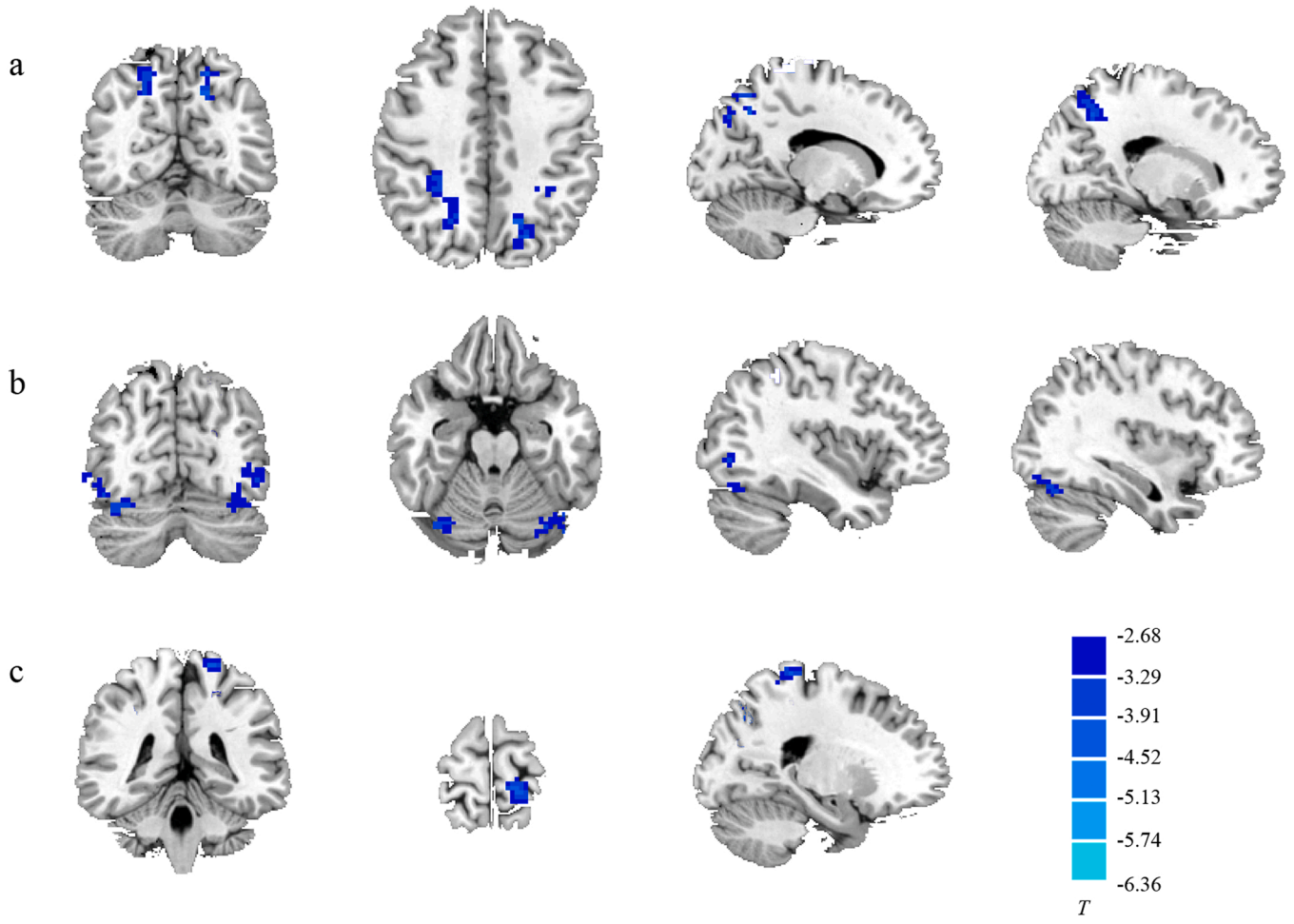

Fig. 2. . RSFC values in the left RMFG – (a) bilateral superior parietal gyrus, (b) bilateral cerebellum, and (c) left postcentral gyrus/paracentral lobule were lower in the HTA group than in the LTA group.

reduction of information transmission efficiency from the ECN to the visual and sensorimotor network, similar to that in patients with depression (Long et al., 2020). The RSFC between the right RMFG and the bilateral thalamus in individuals with HTA was higher rather than lower than that in individuals with LTA. In populations with normal cognitive function, a close association was found between the prefrontal cortex and the thalamus in structure and function (Bonelli & Cummings, 2007; Le Reste, Haegelen, Gibaud, Moreau, & Morandi, 2016), and regulated executive function (Bonelli & Cummings, 2007; Thakkar et al., 2014). Previous studies reported that patients with short-term memory deficits (Voets et al., 2015) or executive control deficits (Orellana & Slachevsky, 2013; C. Zhang et al., 2019) experienced an increased RSFC between the prefrontal cortex and the thalamus. Although the impairment of cognitive function could not be compensated, Voets et al. (2015) believed that the enhancement of prefrontal-thalamic functional connectivity may reflect the remodeling of the damaged pathway of epileptic diffusion change. Similarly, individuals with HTA also exhibited decreased cognitive functioning such as attention and memory (Wei et al., 2021; Zhang & Zhou, 2015). Therefore, the increased RSFC between the right RMFG and the bilateral thalamus in individuals with HTA might reflect the remodeling of their brain-damaged pathways.

This study had some limitations. First, although the participants' demographic variables in each group were controlled as much as possible, a mismatch of age and sex ratio existed between the two groups, and therefore, caution was used when drawing conclusions. Second, a cross-sectional experimental design was adopted in this study, and causal inference could not be carried out. Finally, although we found that the LGI and RSFC values were correlated with TA severity (for example, the LGI in the left RMFG was significantly correlated with TAS scores, $r = -.40$, $p = .045$) in the HTA group, the correlation was not significant after multiple comparison correction. Therefore, in the future, we should further increase the sample size to verify the relationship between the abnormality of brain structure and functional connectivity and the severity of symptoms.

5. Conclusions

Using the surface-based LGI indicator, we investigated the differences in cortical folding and corresponding RSFC between individuals with HTA and LTA. We observed a reduced LGI for the ECN and the visual cortex, a reduced RSFC within the ECN, between the ECN and the visual network, between the ECN and the sensorimotor network, and also an increased RSFC between the right RMFG and the bilateral thalamus in individuals with HTA. These brain structural and functional abnormalities might be the neural basis for the unreasonable cognitive model and maladaptive emotional process in individuals with HTA. Our findings might provide neuroimaging evidence in support of structural and functional abnormalities in individuals with HTA and improve our understanding of the neurobiological underpinnings of TA.

Role of the funding source

The funders had no role in the study design, conduct of the study; in the collection, management, analysis and interpretation of the data; or

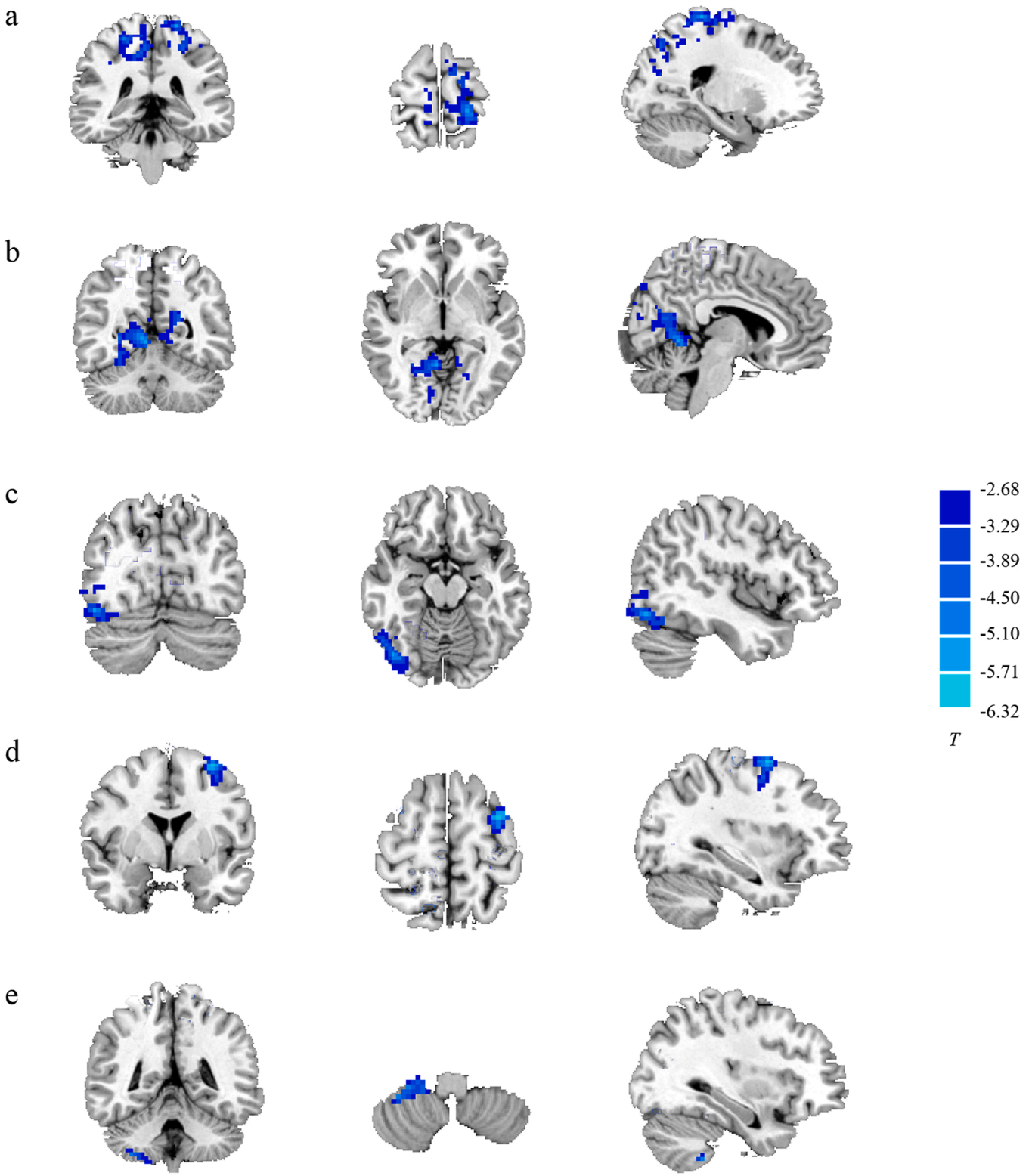

Fig. 3. . RSFC values in the left ITG – (a) bilateral postcentral gyrus, (b) right lingual gyrus, (c) right inferior occipital gyrus, (d) left precentral gyrus, and (e) right cerebellum were lower in the HTA group than in the LTA group.

in the preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Ethical standards

All of the procedures involving human participants were performed in accordance with the ethical standards of the institutional or national research committee and the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

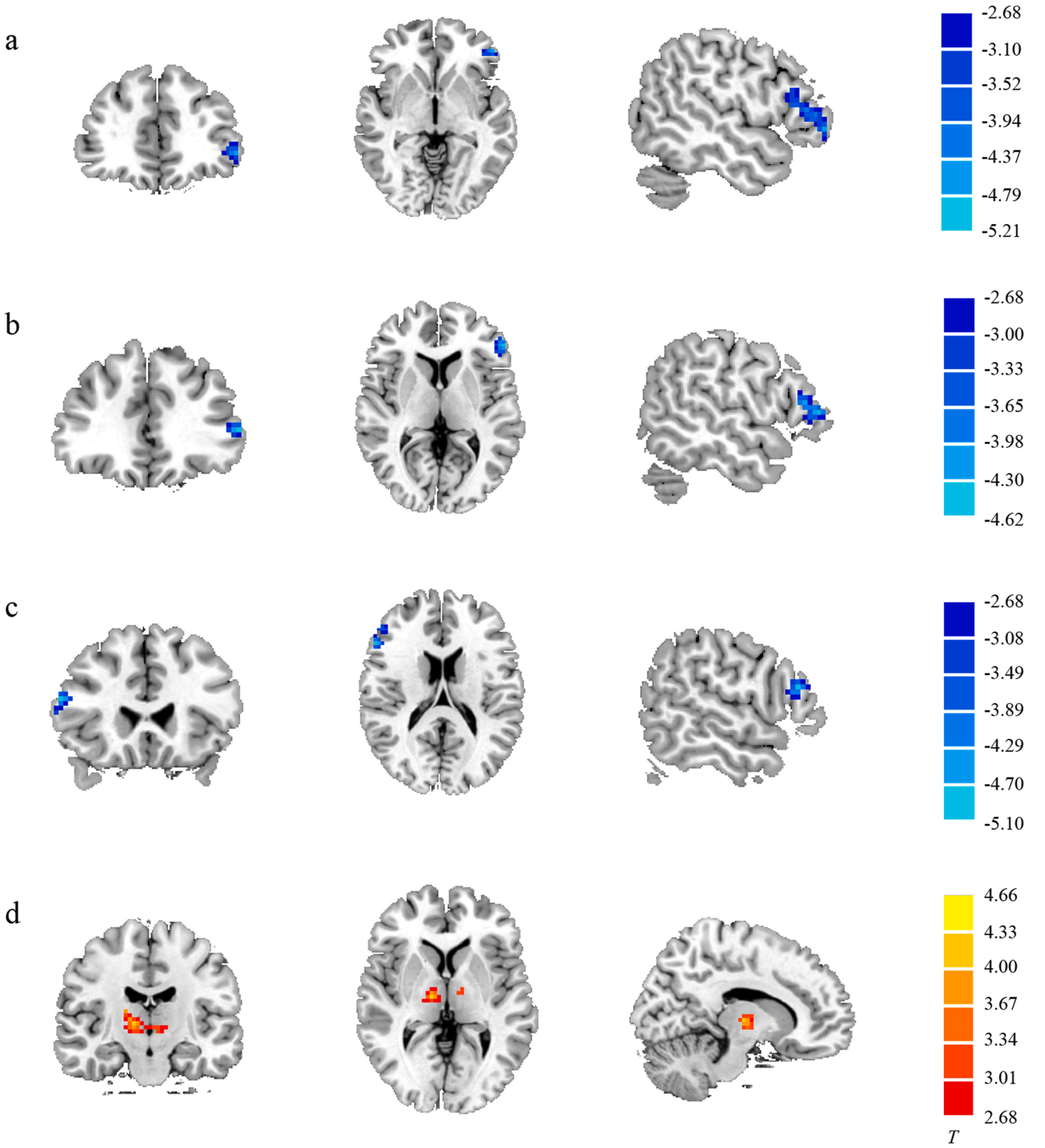

Fig. 4. . RSFC values in the (a) left CMFG–left orbital inferior temporal gyrus, (b) left STG–left triangle inferior frontal gyrus, and (c) right LOG–right triangle inferior frontal gyrus were lower in the HTA group than in the LTA group, while the RESF value of the (d) right RMFG–bilateral thalamus was higher in the HTA group than that in the LTA group.

CRediT authorship contribution statement

Hou Lulu: Conceptualization, Methodology, Formal analysis, Visualization, Writing – original draft. **Zhang Wenpei:** Investigation. **Huang Qiong:** Investigation. **Zhou Renlai:** Conceptualization, Supervision, Funding acquisition, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationshipsthat could have appeared to influence the work reported in this paper.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request after completing a formal data sharing agreement.

Acknowledgements

This study was supported by the Fundamental Research Funds for the Central Universities [2020300048] and Nanjing Institute of Minor Mental Health Research [2020ZK-ZK05]. The authors would like to express their gratitude for the support of these projects.

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