An amygdala-centered effective connectivity network in trait anxiety

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Abstract

Previous studies have established that the amygdala plays an important role in trait anxiety. However, there remains limited knowledge regarding the changes in amygdala-centered effective connectivity network associated with this trait. The current study employed the Granger Causal analysis to investigate the directional connectivity patterns involving the amygdala in relation to trait anxiety in a large cohort of young adults. The results revealed a negative association between trait anxiety scores and the Granger causality from the left middle frontal gyrus and right superior frontal gyrus to the right amygdala. Conversely, higher trait anxiety levels were found to be associated with increased effective connectivity from the left amygdala to the left hippocampus. Moreover, the right superior frontal cortex-to-right amygdala Granger causality partly and negatively mediated the positive correlation between the left amygdala-to-left hippocampus Granger causality and trait anxiety. These results demonstrated the significance of the prefrontal cortex-amygdala-hippocampus neural circuitry in the neurobiological mechanisms underlying trait anxiety. Our findings might contribute to the supervision and mitigation of the potential transition from high trait anxiety to pathological states.

Introduction

Anxiety, a prevalent emotional state, was characterized by persistent experiences of worry, apprehension, fear, and heightened arousal (C. D. Spielberger, 1983; Tian et al., 2016; Tovote, Fadok, & Lüthi, 2015; Xu et al., 2019). Individuals with high trait anxiety demonstrated a greater inclination towards potential internal or external threat stimuli, encountered difficulties in relaxation, and exhibited increased vulnerability to anxiety-related disorders such as generalized anxiety disorder (GAD), major depressive disorder (MDD), and social anxiety disorder (SAD) (Cremers et al., 2010; Grupe & Nitschke, 2013; Huo, Zhang, Seger, Feng, & Chen, 2020; Modi, Kumar, Kumar, & Khushu, 2015; Xu et al., 2019).

It had been confirmed by numerous neuroimaging studies that the amygdala, a critical brain region for emotional processes, played a significant role in the functional and structural neural substrates of anxiety (Ironside et al., 2019; Kim, Loucks, et al., 2011; Phelps & LeDoux, 2005). Converging evidence highlighted a robust association between the amygdala and trait anxiety during the detection and processing of threatening stimuli (Etkin et al., 2004; Günther et al., 2020; Hyde, Gorka, Manuck, & Hariri, 2011). For example, the participants with higher trait anxiety scores exhibited more increased amygdala activation in response to masked fearful faces (Günther et al., 2020). Moreover, at the structural level, studies revealed a positive relationship between amygdala volume and trait anxiety in healthy adults and children (Baur, Hänggi, & Jäncke, 2012; Qin et al., 2014). Notably, patients with GAD also showed increased amygdala volumes compared to control groups, consistent with findings observed in healthy populations (Schienle, Ebner, Schäfer, & neuroscience, 2011).

The amygdala-associated neural network, encompassing the prefrontal cortex (PFC) and hippocampus, had also been consistently implicated in anxiety (Kim & Whalen, 2009; Mah, Szabuniewicz, & Fiocco, 2016). A previous study found that trait anxiety was associated with functional connectivity alterations

within the superior frontal gyrus, underscoring the significance of the PFC's role in anxiety (Saviola et al., 2020). What was more important, the efficacy of top-down inhibition exerted by the PFC on the amygdala, regulating emotional processes through their functional and structural connectivity, appeared to be attenuated in individuals with high trait anxiety and in those diagnosed with anxiety-related disorders (Amaral & dysfunction, 1992; Burghy et al., 2012; Ghashghaei, Hilgetag, & Barbas, 2007; Kim, Gee, Loucks, Davis, & Whalen, 2011; Kim & Whalen, 2009; Porta-Casteràs et al., 2020). For instance, Kim and Whalen reported a negative correlation between trait anxiety scores and the strength of white matter pathways connecting the amygdala and PFC (Kim & Whalen, 2009). Furthermore, compared to healthy controls, SAD patients exhibited more negative connectivity between the amygdala and PFC compared to healthy controls, and this aberrant connectivity was found to be associated with the severity of symptoms, indicative of compromised automatic recruitment of the PFC for regulatory functions in SAD (Young et al., 2017). In addition, by using Granger causal analysis (GCA), Dong et al. found that the middle frontal cortex exhibited a significant direct inhibitory influence on the amygdala in healthy controls, while this inhibitory influence was markedly attenuated in individuals diagnosed with GAD (Dong et al., 2019). Consequently, this study provided empirical evidence regarding the anxiety-related alterations in effective connectivity between the amygdala and PFC specifically. Importantly, it is noteworthy that, to the best of our knowledge, no previous investigations had examined the alterations in amygdala-PFC effective connectivity in relation to trait anxiety among healthy adults.

The hippocampus was another significant brain structure within the amygdala-centered network associated with anxiety (Mah et al., 2016). Extensive research in both rodents and humans had indicated its involvement not only in memory processes but also in anxiety (Bannerman et al., 2004; Lau et al., 2012; MacMillan et al., 2003; Mah et al., 2016; Satpute, Mumford, Naliboff, & Poldrack, 2012). For instance, Satpute et al. demonstrated a significant correlation between trait anxiety and activation of the posterior hippocampus (Satpute et al., 2012). Furthermore, individuals with GAD had been found to exhibit reduced hippocampal volume compared to healthy controls (Alper et al., 2023; Bremner et al., 2000; Gray, Müller, Eickhoff, & Fox, 2020). In contrast to the amygdala's primary role in processing cues that predicted threatening or aversive stimuli, the hippocampus was crucial in processing contextual information related to threat (Alexandra Kredlow, Fenster, Laurent, Ressler, & Phelps, 2022). The interaction between these two brain regions allowed individuals to effectively discriminate between safe and threatening stimuli, facilitating appropriate responses (Mah et al., 2016; Phillips & LeDoux, 1992; VanElzakker et al., 2014). However, any disruption in the delicate balance of neural circuits could result in significant dysfunction in the processing and expression of anxiety. For instance, an excessive information flow from the amygdala to the hippocampus had been observed to induce anxiety-related behaviors in mice (Felix-Ortiz et al., 2013; Felix-Ortiz & Tye, 2014; J.-Y. Zhang et al., 2019). Nonetheless, limited knowledge existed regarding alterations in the effective connectivity between the amygdala and hippocampus associated with trait anxiety in humans.

In the present study, we employed the GCA using the amygdala as seed region, in conjunction with multiple regression analysis, to examine alterations in the causal connectivity network between the amygdala, PFC and hippocampus associated with trait anxiety in a large cohort of young adults. The

GCA, a hypothesis-free approach widely utilized in neuroimaging research, had been employed to delineate effective connectivity patterns of the amygdala in anxiety-related disorders (Dong et al., 2019; K. Friston, Moran, & Seth, 2013; Liao et al., 2010; Seth, Barrett, & Barnett, 2015; Shan et al., 2023). Building upon previous investigations (Dong et al., 2019; Felix-Ortiz & Tye, 2014), we hypothesized a negative association between the effective connectivity from the PFC to the amygdala and trait anxiety, while postulating a positive correlation between amygdala-to-hippocampus connectivity and trait anxiety.

Materials and Methods

Participants

Four hundred and forty-four healthy college students (228 females, mean age ± SD = 19.991 ± 1.118) participated in the study as part of a longitudinal study exploring the relationships between brain imaging and mental health (Liu et al., 2017). None of them reported any history of major medical, psychiatric, or neurological diseases. Twenty participants were excluded due to the overall head motion was above 2.5 mm in translation and 2.5° in rotation, finally leaving 424 participants for subsequent analysis. All participants provided written informed consent according to protocols approved by the Southwest University Brain Imaging Center Institutional Review Board.

Trait Anxiety Assessment

To measure participants' anxiety score, the Chinese version of State Trait Anxiety Inventory (STAI) was used. The STAI contained 40 items for measuring state anxiety (A-State) and trait anxiety (A-Trait) (Shek & Assessment, 1988; C. D. Spielberger, 1983 e. o. p. Spielberger, 2010). The A-Trait scale we used consisted of 20 items that required individuals to report the generally feelings. Each item was rated on a 4-point intensity scale from 1 (not at all) to 4 (very much so). The Chinese version of the STAI has been widely used in previous studies with documented validity and reliability (Han et al., 2020; Tian et al., 2016).

Image Data Acquisition

Brain images were obtained with a 3-Tesla Trio MRI scanner (Siemens) at the Brain Imaging Center at Southwest University. T1-weighted images were acquired with the following parameters: repetition time = 1900 ms, echo time = 2.52 ms, field of view = 256 × 256 mm^2, flip angle = 9˚, matrix size = 256 × 256, and 1 mm^3 isotropic voxel. T2*-weighted echo-planar images (EPI) were obtained with blood oxygenation level-dependent (BOLD) contrast. Thirty-two axial slices covering the whole brain were acquired with TR = 2000 ms, TE = 30 ms, flip angle = 90^o, field of view = 220 × 220 mm, matrix size = 64 × 64, in-plane voxel size = 3.4 × 3.4 mm, and slice thickness = 3 mm with 1 mm gap. Slice scanning order was ascending interleaved. A total of 242 images were acquired for the resting state scan. During the resting state scanning, all participants were requested to close their eyes but keep awake.

Imaging Preprocessing

The fMRI data were preprocessed and analyzed using Statistical Parametric Mapping version 8 (SPM8, Wellcome Department of Imaging Neuroscience, University College London, U.K.) and Data Processing & Analysis for (Resting-State) Brain Imaging (DPABI; http://rfmri.org/DPABI) (Yan, Wang, Zuo, & Zang, 2016). After discarding the first 10 volumes, the remaining 232 fMRI volumes were first slice-time corrected and later head-motion corrected using a least squares approach and a 24-parameter autoregressive model (Friston 24-parameter model) (K. J. Friston, Williams, Howard, Frackowiak, & Turner, 1996). The 24 parameters included six head motion parameters, six head motion parameters one-time point before, and the 12 corresponding squared items. The participants whose head motion exceeded 2.5 mm in translation or 2.5° in rotation were excluded from following analyses. We further calculated frame-wise displacement (FD), which indexed volume-to-volume changes in head position (Power et al., 2014), and used the mean FD as a regressor in further analyses to control head motion better. Subsequently, T1-weighted and functional images were reoriented by hand to optimize alignment for co-registration, segmentation, and normalization (Wang et al., 2017). Individual T1-weighted images were co-registered to the mean motion-corrected functional image. The resulting aligned images were then segmented into grey matter, white matter, and cerebrospinal fluid (CSF). Next, registered images were spatially normalized to Montreal Neurological Institute (MNI) template (resampling voxel size = 3 mm ×3mm × 3 mm), spatially smoothed with a 6 mm FWHM Gaussian filter, and temporally band-pass filtered into 0.01–0.1 Hz to reduce the effect of very low-frequency drift and high-frequency physiological noise. To remove the nuisance signal, the 24 head-motion parameters, CSF, and white matter were regressed out.

Granger Causal Analysis (GCA)

GCA Processing

The seeds for GCA were left and right amygdala, which were defined using the corresponding AAL mask (Tzourio-Mazoyer, Hervé, & Mazoyer, 2007). We calculated the voxel-wise bivariate coefficient GCA by using the REST-GCA in the REST toolbox (http://www.restfmri.net; (Song et al., 2011). We estimated the Granger causal effects between the reference time series of the seed regions (bilateral amygdala) and the time series of each voxel within the whole brain. Two analyses were conducted to explore voxel-wise GCA: seed-to-whole-brain and whole-brain-to-seed. The seed-to-whole-brain analysis explored the driving or inhibitory effects of seeds on other voxels in the brain, whereas the whole-brain-to-seed analysis explored the excitatory or depressive effects of other voxels on the seeds (Hamilton, Chen, Thomason, Schwartz, & Gotlib, 2011; Ji et al., 2013).

After obtaining the bivariate voxel-wise GCA maps of each seed ROI for each participant, we adopted multiple regression analyses to explore the amygdala-centered effective connectivity network that was related with trait anxiety, using the GCA maps as dependent variable and trait anxiety score as independent variable. For reported analyses, an uncorrected voxel threshold of $p < 0.005$ followed by a family-wise error (FWE) corrected threshold of $p < 0.05$ using small volume correction (SVC) was set. The

ROIs for SVC included the bilateral hippocampus and prefrontal cortex (i.e., SFG and MFG). The ROIs were defined using the corresponding AAL mask (Tzourio-Mazoyer et al., 2007). These brain regions were structurally and functionally connected to the amygdala and constituted a neural network involved in the processing and modulation of anxiety (Dong et al., 2019; Felix-Ortiz et al., 2013; Satpute et al., 2012; Saviola et al., 2020). The age, gender and mean FD value were included as regressors in reported analyses

Mediation Analysis

We used a mediation analysis to further examine whether the information flow from PFC (i.e., SFG and MFG) to amygdala could account for the association between the amygdala-to-hippocampus effective connectivity and trait anxiety. The mediation analysis was conducted using the PROCESS macro Model 4 within SPSS 20.0 (Preacher & Hayes, 2008). For this study, effective connectivity between the amygdala and hippocampus was the independent variable, the trait anxiety score was the dependent variable, and SFG/MFG-to-amygdala effective connectivity was the potential mediator, with age, gender, and mean FD included as covariates. The non-parametric bootstrapping analysis was utilized to test the significance of indirect effect. If 95% bias-corrected bootstrap confidence intervals (CI) estimated from 5000 bootstrap samples did not include zero, the mediation effects were considered statistically significant (Yin et al., 2020; H. Zhang, Zhao, Zou, Liu, & Gan, 2021).

Results

GCA Results

Seed-to-Whole-Brain Analysis

For left amygdala seed, we carried out a multiple regression analysis using the GCA maps as dependent variable and trait anxiety score as independent variable. Results revealed a positive association in the left hippocampus ([-36 -12 − 27], voxel = 6, $p = 0.004$, svc) (Figure. 1A), i.e., the higher trait anxiety score was for an individual, the more increase in Granger causality from left amygdala to left hippocampus. For right amygdala see, no significant differences in the brain regions of interest were found (all ps > 0.05).

Whole-Brain-to-Seed Analysis

For the whole-brain-to-seed GCA maps, we also conducted multiple regression analyses by using the trait anxiety score as independent variable. With the right amygdala seed, results showed negative correlations in the left middle frontal gyrus ([-27 12 42], voxel = 10, $p = 0.048$, svc) and the right superior frontal gyrus ([9 39 60], voxel = 38, $p = 0.047$, svc) (Figure. 1B), i.e., participants who had higher trait anxiety score exhibited decreased Granger causality from left MFG and right SFG to right amygdala. There were no significant differences in the brain regions of interest for the left amygdala seed (all ps > 0.05).

Mediation Analysis Results

To examine whether the positive correlation between left amygdala-to-left hippocampus Granger causality and trait anxiety score was mediated through the Granger causality form prefrontal cortex (i.e., MFG and SFG) to amygdala, we conducted mediation analyses. The results showed the right SFG-to-right amygdala Granger causality partly mediated the influence of the left amygdala-to-left hippocampus Granger causality on the trait anxiety score (indirect effect = 2.829; $p = 0.029$) (Fig. 2). Namely, the total effect of left amygdala-to-left hippocampus Granger causality on trait anxiety score was significant ($\beta c = 23.164$, $p < 0.001$), but after taking the mediation effect of right SFG-to-right amygdala Granger causality into consideration, the remaining direct effect was reduced ($\beta c' = 19.558$, $p = 0.006$), although it was still significant. No significant mediation effect was found for the left MFG-to-right amygdala Granger causality (indirect effect = 0.778; $p = 0.447$).

Discussion

We employed the GCA to investigate the alteration of amygdala-related effective connectivity associated with trait anxiety. The results showed that the Granger causality from left MFG and right SFG to right amygdala was negatively associated with the trait anxiety scores. Specifically, participants with higher trait anxiety exhibited diminished information flow from the left MFG and right SFG towards the right amygdala. Conversely, higher trait anxiety was associated with heightened effective connectivity from the left amygdala to the left hippocampus. More interestingly, the right SFG-to-right amygdala Granger causality partly and negatively mediated the positive correlation between the left amygdala-to-left hippocampus Granger causality and the trait anxiety.

As previously stated in the introduction section, the amygdala and its related brain network had been recognized as crucial components in the neurobiology of anxiety (Günther et al., 2020; Kim, Loucks, et al., 2011; Qin et al., 2014). The present study, demonstrating a negative correlation between the left MFG/right SFG-to-right amygdala Granger causality and trait anxiety, was consistent with numerous prior studies (Burghy et al., 2012; De Witte, Mueller, & Behavior, 2017; Kim, Gee, et al., 2011; Kim & Whalen, 2009; Yoo, Park, Kim, & Behavior, 2022). For example, Kim et al. reported a negative association between the amygdala and the ventral medial prefrontal cortex in individuals with high anxiety during a resting state, while a positive correlation between these brain structures was observed in individuals with low anxiety levels (Kim, Gee, et al., 2011). Additionally, Yoo et al. demonstrated that a negative structural network involving the amygdala-PFC circuitry could predict trait anxiety in young adults (Yoo et al., 2022). In contrast to these findings, however, some authors reported a positive correlation between amygdala-PFC connectivity and trait anxiety (Modi et al., 2013; Montag, Reuter, Weber, Markett, & Schoene-Bake, 2012). We speculated that these contradictory findings might stem not only from age and gender differences across the studies (Montag et al., 2012; Yoo et al., 2022), but also from the inherent limitation of the functional connectivity analysis, namely the ambiguity of connectivity direction. To compensate for this, some previous researchers adopted the GCA to investigate the changes in effective connectivity between the amygdala and PFC in patients with anxiety-related disorders (Dong et al., 2019; Liao et al., 2010; Qiao et al., 2017). In comparison to healthy controls, patients with GAD exhibited weakened information flow from the MFG to the amygdala, indicating disrupted top-down regulation from the PFC

to the amygdala (Dong et al., 2019; Qiao et al., 2017). Similar findings were observed in patients with SAD, characterized by decreased effective connectivity from the SFG to the amygdala (Liao et al., 2010). Our results aligned with these prior investigations and further expanded upon them by demonstrating, for the first time, the high trait anxiety-induced impairment of PFC downregulation on the amygdala in a large cohort of healthy adults.

Extensive studies in both rodent and human had consistently demonstrated the critical role of the hippocampus in normal and pathological anxiety (Deji et al., 2022; Ghasemi et al., 2022; Mah et al., 2016; Satpute et al., 2012). The hippocampus assumed a crucial role in processing contextual information associated with threat, working in conjunction with the amygdala to enable accurate discrimination between safe and threatening stimuli, consequently leading to appropriate behavioral responses (Mah et al., 2016; VanElzakker et al., 2014). However, an excessive inputs from the amygdala to the hippocampus has the potential to disrupt the delicate balance of neural circuits, resulting in the induction of anxious behaviors (Felix-Ortiz et al., 2013; Felix-Ortiz & Tye, 2014). For example, through the utilization of optogenetic methodologies, Felix-Ortiz et al. observed that the stimulation of amygdala axon terminals within the hippocampus was concomitant with heightened anxious behaviors. Conversely, inhibition of the axon terminals resulted in reduction of anxiety-related behaviors, implying the significance of information flow from the amygdala to the hippocampus in the process of anxiety (Felix-Ortiz et al., 2013). Our results were consistent with the findings of rodent studies, revealing for the first time an augmented effective connectivity from the amygdala to the hippocampus in individuals with high trait anxiety. Notably, these results might shed light on the underlying mechanisms contributing to the tendency of individuals with high trait anxiety to perceive external and internal stimuli as threatening, despite their innocuous nature (Knowles & Olatunji, 2020). We speculated that the excessive information flow from the amygdala to the hippocampus impaired the ability of individuals with high anxiety to accurately discriminate between safe and threatening stimuli. Furthermore, the mediation analysis results revealed that the information flow from the SFG to the amygdala partially and inversely mediated the positive association between the amygdala-to-hippocampus effective connectivity and trait anxiety scores. Specifically, decreased top-down inhibition from the SFG to the amygdala corresponded to a stronger relationship between effective connectivity from the amygdala to the hippocampus and trait anxiety. These findings demonstrated the significance of the PFC-amygdala-hippocampus neural circuitry in the neurobiological mechanisms involved in anxiety processes.

In summary, this study provided evidence of an association between trait anxiety and the PFC-amygdala-hippocampus circuit. Sustained research indicated that trait anxiety served as a significant vulnerability factor for anxiety-related psychopathology (Andrews, 1991; Knowles & Olatunji, 2020). Consequently, investigating the neural mechanisms underlying trait anxiety was of paramount importance, as it contributed to a deeper understanding of this trait and aided in the supervision and mitigation of its potential progression into pathological conditions.

Declarations

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Compliance with ethical standards

Conflict of interest The authors do not have any conflicts of interest to disclose.

Ethical standards This research involving human participants was approved by the Southwest University Brain Imaging Center Institutional Review Board.

Informed consent All participants provided written informed consent.

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Figures

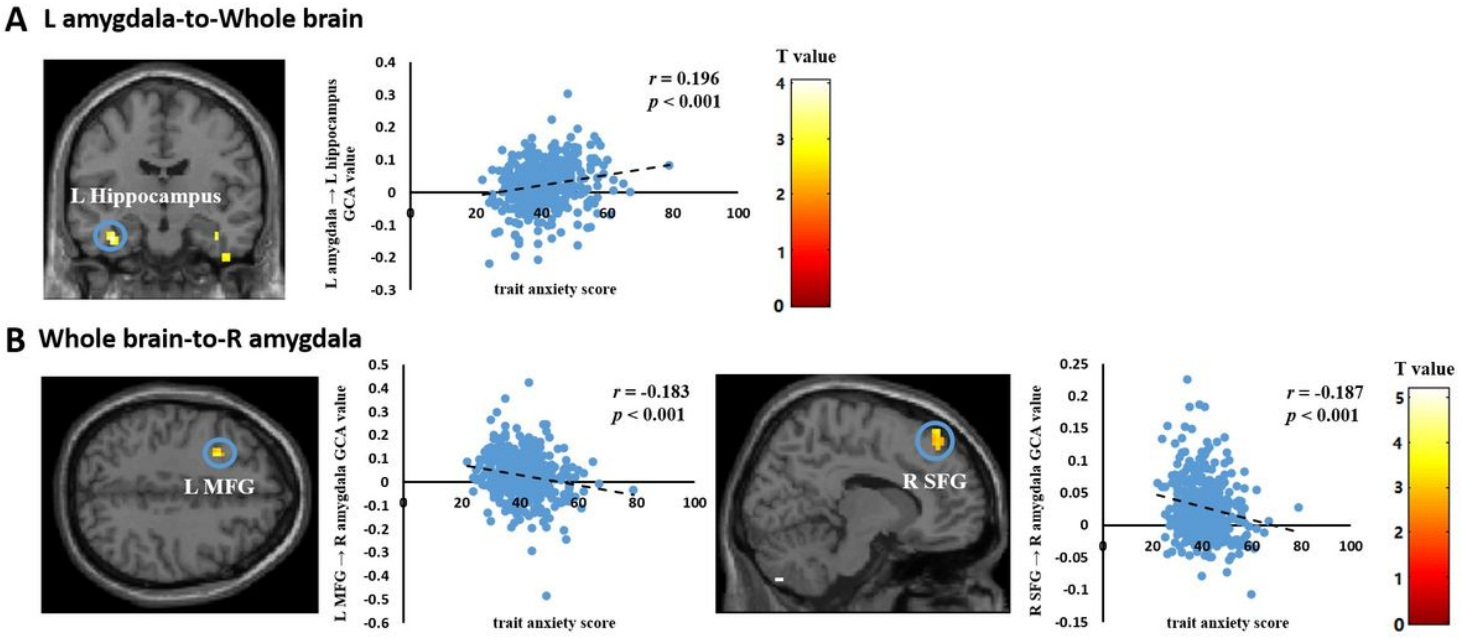

Figure 1

(A) The results of Seed-to-Whole-Brain analysis. The Granger causality from left amygdala to left hippocampus was positively associated with the trait anxiety score; (B) The results of Whole-Brain-to-Seed analysis. The Granger causality from left MFG (left side of the figure) and right SFG (right side of the figure) to right amygdala was negatively associated with the trait anxiety score. L, left; R, right; MFG, middle frontal cortex; SFG, superior frontal cortex; GCA, Granger causal analysis. $p < 0.05$ (SVC corrected).

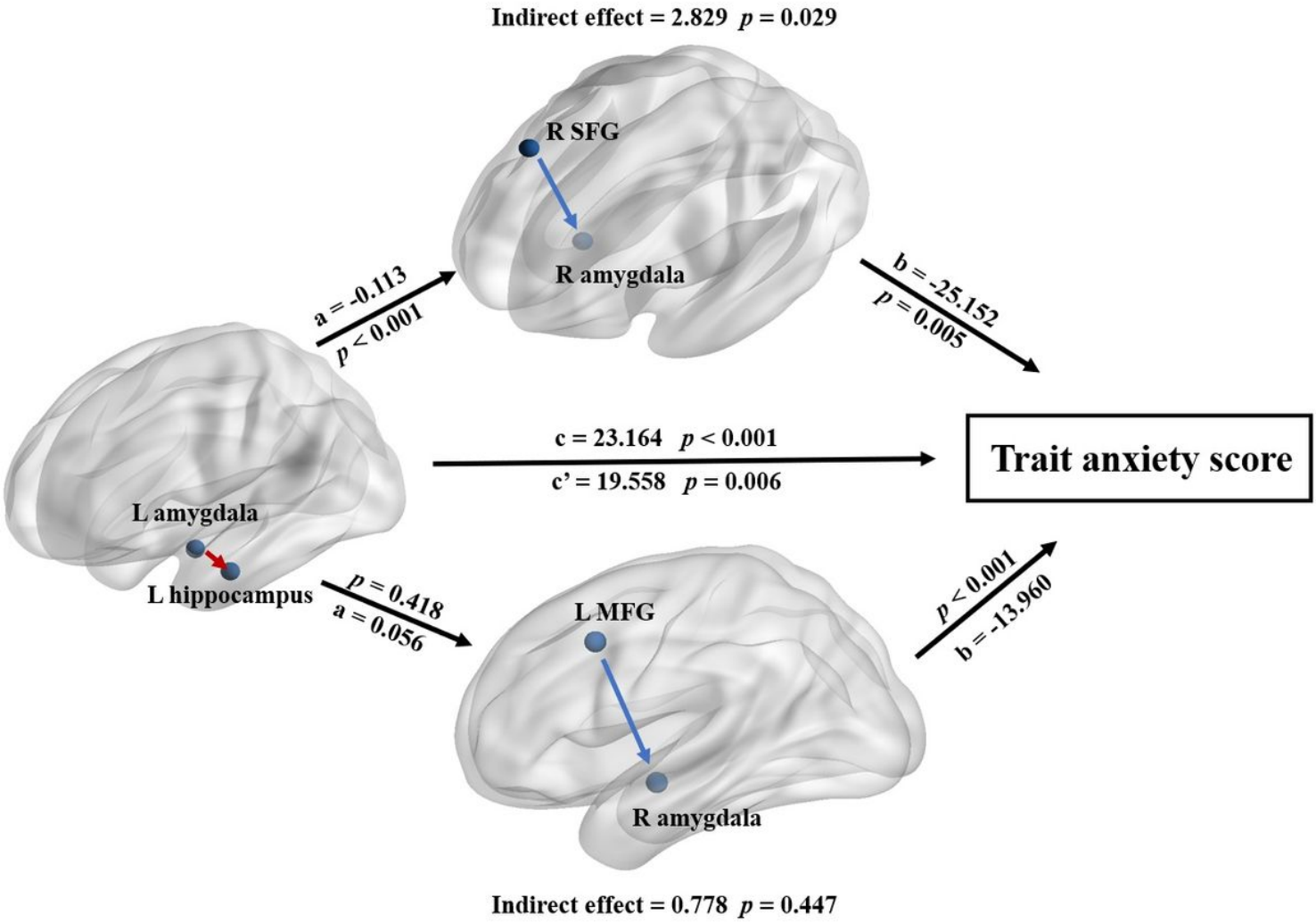

Figure 2

The results of mediation analysis. The right SFG-to-right amygdala Granger causality partly and negatively mediated the positive association between the left amygdala-to-left hippocampus Granger causality and the trait anxiety.L, left; R, right; MFG, middle frontal cortex; SFG, superior frontal cortex; red arrow, positive correlation with trait anxiety; blue arrow, negative correlation with trait anxiety.