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Aberrant resting-state functional connectivity in medication-naïve generalized anxiety disorder: a whole-brain exploratory fMRI study

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Background: Generalized Anxiety Disorder (GAD), characterized by excessive worry and somatic symptoms. Although neuroimaging studies have identified alterations in functional connectivity (FC), structural integrity, and neural activation in GAD, most include medicated or psychotherapy-treated patients, limiting insights into the neurobiology of the untreated state. This study investigated resting-state FC (rsFC) abnormalities in medication-naïve GAD patients using a whole-brain, data-driven approach.

Methods: In this cross-sectional study, medication-naïve GAD patients ($n = 85$) and HCs ($n = 82$) underwent rs-fMRI at Guang'anmen Hospital on a Siemens 3.0T scanner. Data were analyzed using CONN toolbox (v22.v2407). After preprocessing, cluster-based rsFC was examined across 9, 453 connections in 138 ROIs (FSL Harvard-Oxford atlas, excl. cerebellum). Clusters correlated with HAMA scores; rsFC for 10 ROI pairs extracted via MATLAB; key ROIs seeded voxel-wise maps in SBC, controlling gender.

Results: Significant group differences emerged in rsFC clusters, centered on connections between the posterior cingulate cortex (PCC) and right supramarginal gyrus (SMG). Compared to HCs, GAD patients exhibited hyper-connectivity in 5 connections and hypo-connectivity in 5 others within these clusters. Four connections showed positive correlations with HAMA scores.

Limitations: The analysis of 9, 354 connections may have reduced statistical power, possibly obscuring additional relevant findings.

Conclusion: This study demonstrates aberrant resting-state functional connectivity in medication-naïve GAD patients, particularly enhanced PCC-SMG rsFC correlated with anxiety severity, suggesting a potential role for interoceptive hypersensitivity in GAD pathophysiology. These findings support the hypothesis of SMG-driven vigilance engaging PCC and mPFC to perpetuate anxiety cycles, warranting future validation with direct interoceptive measures and highlighting neural targets for interventions.

KEYWORDS

default mode network, functional connectivity, functional magnetic resonance imaging, generalized anxiety disorder, posterior cingulate cortex, resting-state fMRI, supramarginal gyrus

1 Introduction

Generalized anxiety disorder (GAD) is defined by persistent, excessive, and uncontrollable worry—lasting for at least 6 months on most days—accompanied by somatic symptoms such as restlessness, fatigue, impaired concentration, irritability, muscle tension, and sleep disturbance (1). With a lifetime prevalence of approximately 6.2% (2), GAD imposes a substantial clinical and socioeconomic burden. Established treatments, such as cognitive behavioral therapy (CBT) (3), mindfulness-based stress reduction (MBSR) (4) and non-invasive brain stimulation (5), effectively reduce symptoms, yet the underlying neural mechanisms remain incompletely understood.

Neuroimaging evidence implicates dysregulated corticolimbic circuitry in the pathophysiology of GAD. Amygdala hyperactivity to threatening stimuli is a hallmark (6, 7), coupled with medial prefrontal cortex (mPFC) and anterior cingulate cortex (ACC) activation during worry (8). This is manifested by reduced functional connectivity (FC) between the amygdala and mPFC/ACC (9). Additional impairments occur in the ventrolateral prefrontal cortex (vlPFC), dorsolateral prefrontal cortex (dlPFC), posterior parietal regions, and amygdala (10), with altered amygdala-prefrontal FC underpinning excessive worry and autonomic dysregulation (11).

Hypothesis-driven analyses target the corticolimbic circuit—encompassing the amygdala, ACC, and prefrontal cortex (PFC)—where abnormal blood-oxygen-level-dependent (BOLD) responses predominate (12). Task-based functional magnetic resonance imaging (fMRI) reveals task-specific alterations in regions like the insula and hippocampus, but is limited by: small sample sizes constrain statistical power (13), and BOLD signal test-retest reliability is poor (14, 15).

Resting-state FC (rsFC) findings in GAD show heterogeneity, often attributable to seed region of interest (ROI) selection (16). Data-driven whole-brain methods address this by minimizing bias, such as through entropy measures like approximate entropy (ApEn) and sample entropy (SampEn) (17), or voxel-based FC strength-cerebral blood flow correlations (18). Integrating rsFC with structural MRI and graph theory further elucidates network and anatomical changes (19). A recent coordinate-based meta-analysis comparing rsFC in GAD and insomnia disorder highlights overlapping abnormalities in prefrontal and limbic regions, underscoring diagnostic overlap and the need for disorder-specific investigations (20).

Structural studies reveal impaired white matter integrity in emotion regulation tracts, including the uncinate fasciculus, inferior fronto-occipital fasciculus, and inferior longitudinal fasciculus (21). Gray matter reductions appear in the left superior temporal gyrus, with severity-linked changes in limbic areas like the lentiform nucleus and striatum (22). However, the ENIGMA Anxiety Working Group meta-analysis ($n = 1112$ GAD patients, 3282 healthy controls) detected no significant differences in cortical

thickness, surface area, or subcortical volumes, indicating subtle or heterogeneous structural effects (23).

Research on first-episode, medication-naïve GAD patients reports negative correlations between left anterior insula-mPFC rsFC and somatic anxiety, though limited by small samples (e.g., $n = 34$ GAD, 30 controls) (24). The present study addresses these gaps with a larger medication-naïve sample to explore whole-brain rsFC abnormalities, employing a data-driven approach to overcome ROI biases and enhance understanding of untreated GAD neurobiology.

2 Materials and methods

2.1 Participants

This cross-sectional study was conducted at Guang'anmen Hospital, China Academy of Chinese Medical Sciences, between September 2022 and September 2024. A total of 94 medication-naïve patients with GAD and 91 healthy controls (HCs) were enrolled. All participants provided written informed consent in accordance with the Declaration of Helsinki. The study protocol was approved by the Institutional Ethics Committee (Approval No. 2022-081-KY).

2.2 Clinical assessment

GAD was diagnosed per the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5; American Psychiatric Association, 2013).

Inclusion criteria were: (1) age 18–69 years; (2) DSM-5 GAD diagnosis; (3) Hamilton Anxiety Rating Scale (HAMA) score ≥ 7 ; (4) right-handedness; (5) stable vital signs, clear consciousness, and adequate communication ability; (6) written informed consent.

Exclusion criteria were: (1) Hamilton Depression Rating Scale (HAM-D-24) score ≥ 8 ; (2) anti-anxiety treatment in the past month; (3) history of alcoholism or substance dependence; (4) severe hepatic or renal insufficiency; (5) unstable vital signs; (6) pregnancy or lactation; (7) left-handedness; (8) MRI-incompatible implants (e.g., cardiac pacemakers, artificial heart valves, cochlear implants); (9) claustrophobia.

Both groups were evaluated for anxiety and depressive symptoms via the HAMA and HAM-D-24 assessments. We screened for psychiatric comorbidities and excluded individuals with recent anti-anxiety/antidepressant use, psychotherapy, or physical therapy.

Demographic and clinical data were analyzed in SPSS version 26.0. Normally distributed continuous variables with homogeneous variance were compared via independent-samples *t*-tests; non-normal variables used the Mann-Whitney *U* test. Categorical variables were assessed with chi-square tests. Significance was set at $p < 0.05$, with $p < 0.01$ denoting high significance.

2.3 MRI data acquisition

Scans were acquired at Guang'anmen Hospital using a Siemens Magnetom Skyra 3.0T scanner with a 20-channel phased-array head coil (Serial Number: 45617). Sequences included: (a) axial T1-weighted imaging (T1WI): repetition time (TR) = 2530 ms, echo time (TE) = 2.98 ms, matrix = 256×256 , field of view (FOV) = 256×256 mm², slice thickness = 1 mm, slice gap = 1 mm, flip angle = 7°, voxel size = $1 \times 1 \times 1$ mm³, 192 slices; (b) resting-state blood-oxygen-level-dependent (BOLD) imaging via gradient echo-echo planar imaging (GE-EPI): TR = 2000 ms, TE = 30 ms, matrix = 64×64 , FOV = 224×224 mm², slice thickness = 3.5 mm, slice gap = 0.6 mm, flip angle = 90°, voxel size = $3.5 \times 3.5 \times 3.5$ mm³, 36 slices.

2.4 Data preprocessing and analysis

Functional MRI (fMRI) data analyses were performed using CONN FC toolbox version 22.v2407 (25) and SPM12 version 25.25.01.02 (RRID: SCR_007037). Preprocessing steps included: 1. head motion correction. 2. slice-timing correction. 3. coregistration of structural and functional images. 4. spatial normalization. 5. scrubbing of timepoints exceeding 0.9 mm framewise displacement or > 5 standard deviations (26). 6. resampling to 2-mm isotropic voxels using the the IXI-549 template (default in CONN) tissue probability map template (27). 7. spatial smoothing with an 8-mm full-width at half-maximum (FWHM) Gaussian kernel. 8. bandpass filtering (0.01–0.08 Hz).

Denoising: Functional MRI (fMRI) data underwent standard denoising procedures involving regression of potential confounding effects characterized by white matter time series (5 CompCor noise components), cerebrospinal fluid time series (5 CompCor noise components), motion parameters with their first-order derivatives (12 factors), scrubbed scans (89 factors), session effects with their first-order derivatives (2 factors), and linear trends within each functional run (2 factors), followed by bandpass frequency filtering of BOLD time series at 0.008–0.09 Hz (28).

First-level analysis estimated region-of-interest-to-region-of-interest (ROI-to-ROI) connectivity matrices (RRC) and seed-based connectivity maps (SBC) based on 156 HCP-ICA networks (29) and Harvard-Oxford atlas ROIs (30). FC was quantified as Fisher-transformed bivariate correlations from weighted generalized linear models (weighted-GLM (31)), modeling BOLD time-series associations per seed-target pair. magnetization transient effects were addressed by weighting scans with a step function convolved with SPM's canonical hemodynamic response function.

Group-level analysis used generalized linear models (GLMs), with first-level connectivity as the dependent variable and group as the independent variable. Voxel-wise tests applied multivariate parametric statistics with random effects and covariance estimates. Cluster-level inference relied on Gaussian random field theory (32), thresholded at voxel-level $p < 0.01$ and cluster-level p -FDR < 0.05 (33).

2.5 ROI-to-ROI analysis

We employed CONN's default FSL Harvard-Oxford atlas (excluding cerebellum), yielding 9, 453 connections across 138 ROIs. rsFC was computed as Fisher z -transformed Pearson correlations from weighted-GLMs. Thresholds were connection-level $p < 0.05$ and cluster-level p -FDR < 0.05, covarying age and sex. Excessive motion (> 0.5 mm framewise displacement) was excluded via quality assurance outputs. SMG-PCC rsFC was specifically examined for clinical correlations.

2.6 rsFC correlation with clinical scores

HAMA assessed GAD severity. rsFC values for 10 predefined ROI pairs (including SMG and PCC) were extracted from CONN first-level Fisher z -transformed matrices using custom MATLAB scripts (n=185 participants). Variables met Shapiro-Wilk normality in SPSS 26.0. Pearson correlations with HAMA controlled for age and sex, with Bonferroni-corrected $p < 0.05$ (for 10 comparisons) to address multiplicity.

2.7 Seed-to-voxel analysis (SBC)

Key ROIs from RRC results served as seeds for voxel-wise maps in CONN's SBC module. Despite seed selection challenges in whole-brain analyses (34), *post-hoc* exploration of identified ROIs facilitated rsFC pattern assessment (35). Group-level GLMs covaried age and sex. Inference used randomization/permutation statistics for contiguous voxel clusters (36), thresholded at voxel-level $p < 0.01$ and cluster-level p -FDR < 0.05 (CONN defaults).

3 Results

3.1 Demographic data and clinical characteristics

We initially recruited 185 participants (94 with GAD, 91 HCs). After excluding 6 GAD patients and 12 HCs for excessive head motion during fMRI (primarily due to scanner noise-induced discomfort, a common source of motion artifacts (37)), the final sample consisted of 167 individuals (85 with GAD, 82 HCs).

The GAD group exhibited a mean HAMA score of 23.7 (SD = 3.7), indicative of moderate-to-severe anxiety, which was significantly higher than that of HCs ($p < 0.001$). Additionally, the GAD group showed significantly elevated Hamilton Depression Rating Scale (HAM-D-24) scores compared to HCs ($p < 0.001$), likely due to anxiety-related items within the HAM-D-24. This elevation did not affect the validity of subsequent statistical analyses.

Demographic and clinical characteristics are presented in Table 1. The GAD group had significantly higher HAMA scores (mean = 23.7, SD = 3.7) than HCs ($p < 0.001$), corresponding to

TABLE 1 Demographic and clinical data of analyzed participants.

Variables	GAD (n = 85)	HC (n = 82)	Z/ χ^2	p
	Mean \pm SD	Mean \pm SD		
Age (years)	44.0 \pm 13.8	42.4 \pm 13.4	0.752	0.512
Sex (male/female)	25/60	38/44	5.092	0.024
Duration (year)	1.2 \pm 0.6			
HAMA	23.7 \pm 3.7	5.0 \pm 2.1	-11.18	< 0.001
HAMD-24	6.7 \pm 2.0	1.9 \pm 1.1	-10.74	< 0.001

Group differences were evaluated using *Mann-Whitney Test* for Age, HAMA and HAMD-24, and *chi-square test* for sex.

moderate-to-severe anxiety levels. HAMD-24 scores were also elevated in the GAD group ($p < 0.001$), likely due to overlapping anxiety items, without impacting subsequent analyses.

3.2 rsFC alterations detected in ROI-to-ROI analysis

Group differences in rsFC emerged at the cluster level between patients with GAD (n = 85) and HCs (n = 82) ($F(4, 162) = 6.6, p < 0.001, p\text{-FDR} = 0.013$), involving two clusters: (1) right supramarginal gyrus (SMG) and right pars opercularis of the inferior frontal gyrus (IFG pars opercularis); (2) posterior cingulate cortex (PCC), medial prefrontal cortex (mPFC), left anterior inferior temporal gyrus (aITG), and left anterior middle temporal gyrus (aMTG). Regions with differential FC in these clusters are detailed in Figure 1 and Table 2.

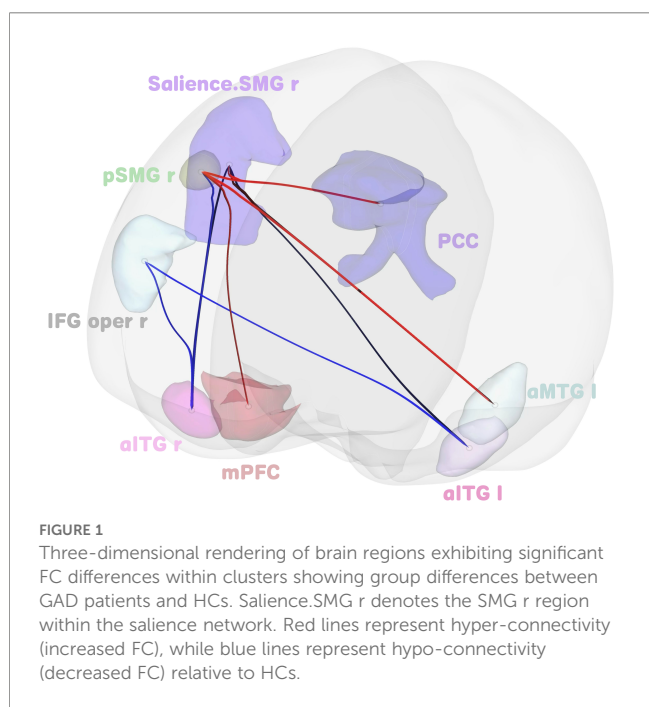


TABLE 2 Cluster and cluster-forming connections with significant difference between GAD and HC.

Cluster	F(4, 162)	p	p-FDR
	6.6	0.001 <	0.012764
Connections		T	p
pSMG r	aITG r	-2.72	0.007298
	aITG l	-2.59	0.010458
	aMTG l	2.51	0.013135
	PCC	2.22	0.027510
IFG oper r	aITG l	-2.26	0.024934
	aITG r	-2.19	0.029903
Salience.SMG r	PCC	2.34	0.020290
	aMTG l	2.29	0.023050
	mPFC	2.13	0.034414
	aITG r	-2.11	0.036113

F = MSB/MSW, representing the ratio of between-group variance (mean square between, MSB) to within-group variance (mean square within, MSW) in analysis of variance (ANOVA) for group-level inference. pSMG, posterior supramarginal gyrus; aITG, anterior inferior temporal gyrus; aMTG, anterior middle temporal gyrus; PCC, posterior cingulate cortex; IFG oper, inferior frontal gyrus, pars opercularis; Salience.SMG, supramarginal gyrus in the salience network; mPFC, medial prefrontal cortex; l, left; r, right.

3.3 Correlation between clinical scores and rsFC

Moderate correlations were identified between HAMA scores and FC (Table 3). Specifically, positive correlations were found for the following connections: pSMG r – aMTG l; pSMG r – PCC; Salience.SMG r – PCC; Salience.SMG r – aMTG l. The correlation analysis and its brain connections are shown in Figure 2.

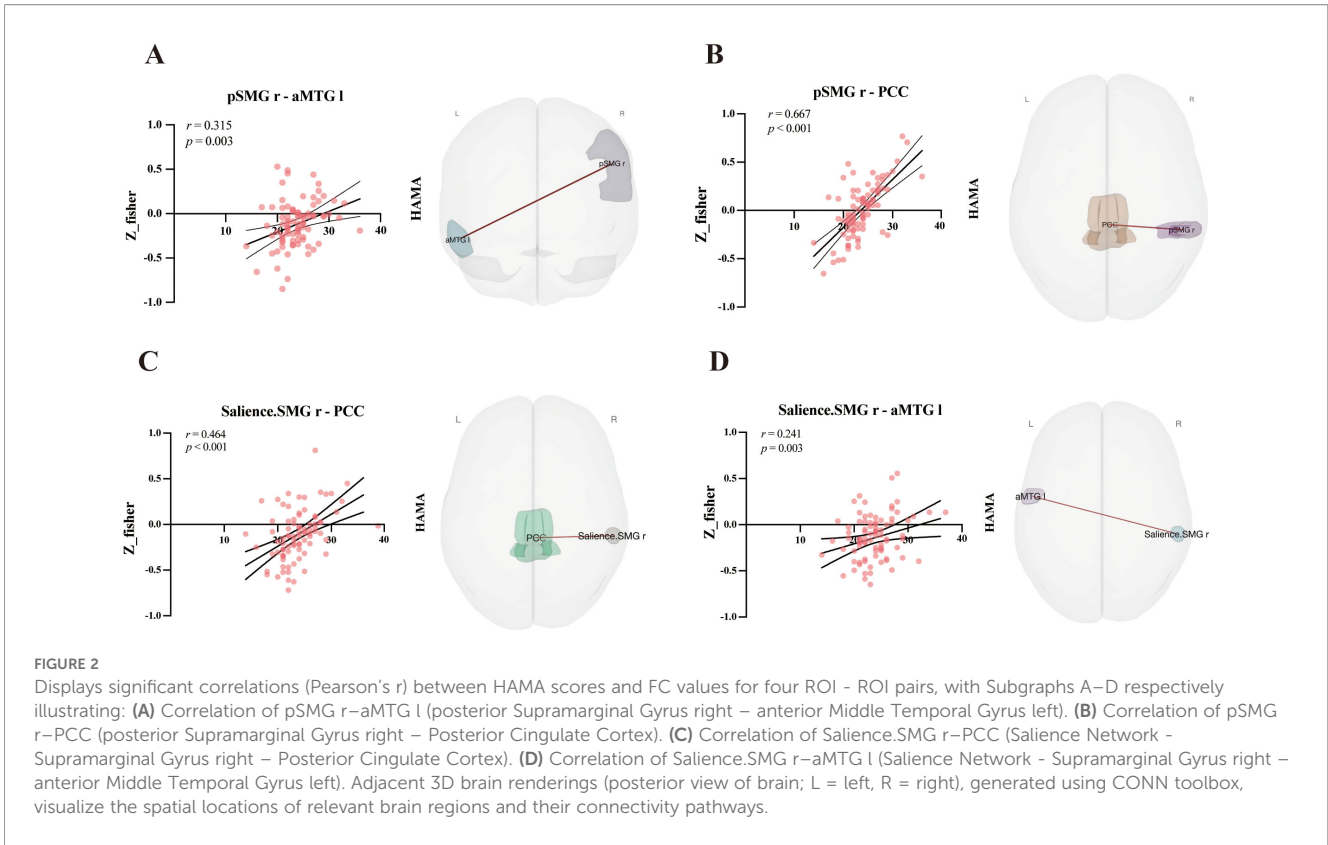
3.4 Seed-based connectivity results

Seed-based connectivity findings are presented in Figure 3. With the PCC as seed, increased rsFC was noted with the right SMG and right prefrontal regions. For the right SMG in the salience network (Salience.SMG r) as seed, increased rsFC occurred in the

TABLE 3 Significant correlations between FC and clinical scores.

Scale	ROI	ROI	r	P
HAMA	pSMG r	PCC	0.667	< 0.001
	pSMG r	aMTG l	0.315	0.003
	Salience.SMG r	PCC	0.464	< 0.001
	Salience.SMG r	aMTG l	0.241	0.003

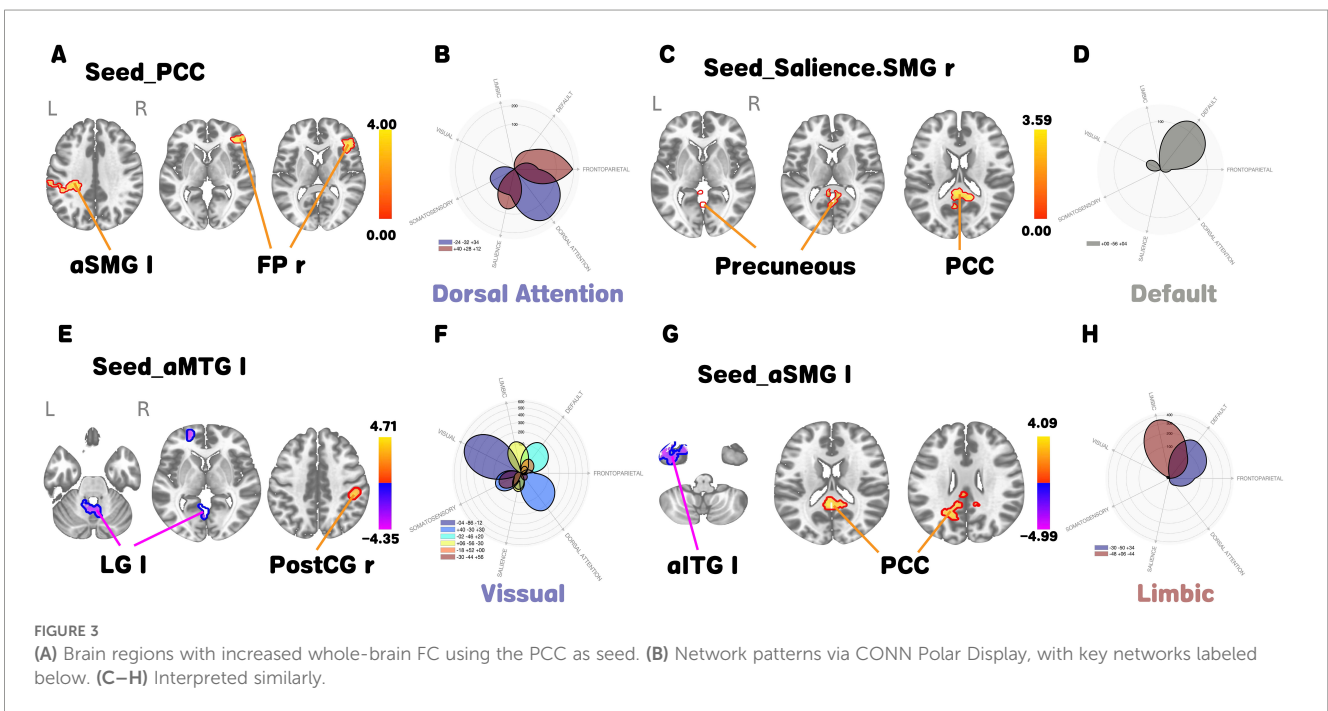
r = Pearson correlation coefficient. HAMA scores and FC z-scores met normality assumptions via Shapiro-Wilk test; associations were evaluated using Pearson correlation with $p < 0.05$ (uncorrected for multiple comparisons). pSMG r and Salience.SMG r show partial spatial overlap, with Salience.SMG r covering a broader area and pSMG r limited to an SMG r subregion (l, left; r, right).



PCC and precuneus. The left anterior middle temporal gyrus (aMTG l) seed showed decreased rsFC with the left lingual gyrus and increased rsFC with the right postcentral gyrus. The left SMG seed revealed decreased rsFC with the left anterior inferior temporal gyrus (aITG l) and increased rsFC with the PCC.

4 Discussion

This study reports novel enhanced rsFC between the right SMG and PCC in medication-naïve patients with generalized anxiety disorder (GAD). This connectivity correlated strongly with



Hamilton Anxiety Rating Scale (HAMA) scores ($r = 0.667, p < 0.001$), implicating SMG - PCC interactions in GAD pathophysiology.

ROI-to-ROI analyses identified increased right SMG-PCC FC, while seed-to-voxel analyses with the PCC as seed revealed further enhancements involving the left anterior SMG and right PFC. The SMG contributes to sensory integration, working memory, and executive control (38). Prior GAD studies indicate SMG abnormalities, such as reduced right SMG-superior parietal gyrus rsFC (39) and elevated local gyrification index in the SMG (40), a marker of psychiatric risk (41). Heightened SMG activity in GAD may result in misattribution of neutral stimuli as threats, intensifying hypervigilance.

The right SMG volume associates with emotion recognition (42), and the SMG supports overcoming emotional egocentricity (43) and mental state inference (44). Increased right SMG-PCC-medial prefrontal cortex (mPFC) FC could signify amplified SMG engagement in anxiety, modulating self-referential and socio-emotional processes through PCC interoception and mPFC cognition. Hemispheric asymmetry is evident: the left SMG focuses on tool use and language (45, 46), whereas our data emphasize right SMG dominance in GAD.

Midline default mode network (DMN) hubs, including the PCC and anterior mPFC, engage in self-referential emotional processing (47). Salience network (SN) -DMN dysconnectivity correlates with anxiety (48), with reduced intra-DMN rsFC involving the PCC and SMG in high-anxiety states (49) and dynamic limbic-prefrontal/DMN decrements in GAD (50). Our observed right SMG-PCC-mPFC hyperconnectivity suggests dysregulated network coupling, fostering threat misinterpretation and sustained vigilance.

Current models posit GAD anxiety stems from disruptions beyond acute fear circuits, encompassing sustained threat, reward, cognitive control, and socio-emotional domains (51). High anxiety involves intensified negative emotion processing, disrupting regulation (52). The right SMG, key for interoceptive awareness and attention, may exaggerate bodily (e.g., heartbeat) or threat signals in GAD, driving hypervigilance (53). The PCC, a DMN core (54, 55), sustains self-referential negative focus, fueling worry cycles. Right SMG-PCC hyperconnectivity may indicate DMN-fronto-parietal network (FPN) misalignment (56), excessive integration of interoceptive/threat cues at rest and amplifying symptoms. These findings are consistent with an interoceptive bias in GAD, where bodily signal hypersensitivity sustains anxiety; in resting-state fMRI, such rsFC may reflect threat attribution to benign cues absent external stimuli.

These findings on enhanced PCC-SMG connectivity could serve as a potential biomarker for identifying GAD subtypes, enabling personalized interventions such as fMRI-guided neurofeedback to normalize interoceptive networks and reduce anxiety symptoms. Furthermore, targeting interoceptive hypersensitivity through therapies like mindfulness-based stress reduction or interoceptive exposure may improve clinical outcomes in medication-naïve patients, bridging neuroimaging insights to real-world treatment strategies.

A key limitation of this study is the absence of direct measures of interoceptive awareness and attention, which constrains precise

inferences about the relationship between supramarginal gyrus (SMG) activation and subjective bodily alertness. Although fMRI data highlight SMG's pivotal role in the somatosensory awareness network (57), and its potential recruitment of the posterior cingulate cortex (PCC) and medial prefrontal cortex (mPFC) in emotional processing (58), these findings remain correlational without behavioral or physiological validation (e.g., heartbeat detection tasks). Future research should incorporate multimodal approaches, such as interoceptive sensitivity scales and heart rate variability monitoring, to confirm SMG-driven vigilance in amplifying anxiety symptoms (59). Moreover, longitudinal designs could elucidate the dynamic recruitment of PCC/mPFC in precipitating anxiety cycles over time (60), informing targeted interventions like interoception-focused cognitive behavioral therapy.

No alterations emerged in typical GAD regions like the amygdala (61, 62), hippocampus (63), ACC (64), or dorsomedial PFC (65). This may arise from our data-driven whole-brain method and exclusive medication-naïve sample, yielding distinct cohort features versus prior work.

The GAD sample showed gender imbalance (60 females, 25 males), covaried in analyses. GAD prevalence is twice as high in females (66), with sex influencing anxiety neurobiology (67–69). Future studies must probe sex effects. Seed-based connectivity power may be limited (70), and broader sample heterogeneity could constrain generalizability (71). Clinically, SMG-PCC rsFC could target interventions like transcranial magnetic stimulation or interoception-focused therapies.

A further limitation concerns illness duration. Although this variable was collected, it was not entered as a covariate in the primary group-level rs-fMRI analyses. In functional connectivity studies of GAD, disease duration has generally shown weaker and less consistent effects compared with structural MRI studies (e.g., gray matter volume loss or cortical thickness reduction). Moreover, self-reported duration in GAD patients is often imprecise and highly variable because of the insidious onset and fluctuating course of symptoms, which reduces its reliability as a quantitative covariate. For these reasons, and consistent with many prior rs-fMRI investigations of anxiety disorders (72–74), we controlled only for age and gender in the main models.

5 Conclusions

In this study, patients with GAD exhibited enhanced rsFC between the posterior cingulate gyrus and the SMG r , which was highly positively correlated with the HAMA scale scores. These findings suggest a potential role for heightened SMG activity in contributing to interoceptive vigilance, potentially engaging the PCC and mPFC in emotional processing that may perpetuate anxiety cycles; however, direct measures of interoceptive awareness (e.g., heartbeat detection tasks) are required to validate this mechanism in future research. This pattern underscores interoceptive hypersensitivity as a potential core feature of GAD, warranting further exploration into its etiological

contributions. Interoceptive sensitivity (75, 76), plays a crucial role in anxiety, and therapeutic approaches targeting interoceptive regulation may become important targets for the treatment of GAD.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Ethics Committee of Guang'anmen Hospital, China Academy of Chinese Medical Sciences. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

YJ: Resources, Conceptualization, Project administration, Software, Writing – original draft, Data curation, Validation, Methodology, Visualization, Formal Analysis, Writing – review & editing, Investigation. TZ: Project administration, Supervision, Software, Writing – review & editing, Resources. WZ: Data curation, Formal Analysis, Writing – review & editing, Investigation, Conceptualization. SL: Visualization, Validation, Resources, Writing – review & editing, Project administration. YC: Funding acquisition, Software, Writing – review & editing, Resources, Formal Analysis. JP: Funding acquisition, Project administration, Resources, Visualization, Formal Analysis, Writing – review & editing. SH: Resources, Writing – original draft, Software, Visualization, Funding acquisition, Conceptualization, Methodology.

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Conflict of interest

The authors declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The authors declared that generative AI was used in the creation of this manuscript. The authors used Grok 4 (<https://grok.com>) to enhance the manuscript's readability and language. All content was subsequently reviewed and edited by the authors, who assume full responsibility for the final publication.

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References

- DeMartini J, Patel G, Fancher TL. Generalized anxiety disorder. *Ann Intern Med.* (2019) 170:ITC49–64. doi: 10.7326/AITC201904020
- Szuhany KL, Simon NM. Anxiety disorders: A review. *JAMA.* (2022) 328:2431–45. doi: 10.1001/jama.2022.22744
- van Dis EAM, van Veen SC, Hagensma MA, Batelaan NM, Bockting CLH, van den Heuvel RM, et al. Long-term outcomes of cognitive behavioral therapy for anxiety-related disorders: A systematic review and meta-analysis. *JAMA Psychiatry.* (2020) 77:768. doi: 10.1001/jamapsychiatry.2020.1242
- Kabat-Zinn J, Massion AO, Kristeller J, Peterson LG, Fletcher KE, Pbert L, et al. Effectiveness of a meditation-based stress reduction program in the treatment of anxiety disorders. *Am J Psychiatry.* (1992) 149:936–43. doi: 10.1176/ajp.149.7.936
- Duan N, Zhang Y, Wang S, Guan J, Ji Y, Huang W, et al. Evaluating the efficacy and acceptability of non-invasive brain stimulation for generalized anxiety disorder: a systematic review and network meta-analysis. *Psychiatry Res Neuroimaging.* (2025) 349:111989. doi: 10.1016/j.pscychresns.2025.111989
- Nitschke JB, Sarinopoulos I, Oathes DJ, Johnstone T, Whalen PJ, Davidson RJ, et al. Anticipatory activation in the amygdala and anterior cingulate in generalized anxiety disorder and prediction of treatment response. *Am J Psychiatry.* (2009) 166:302–10. doi: 10.1176/appi.ajp.2008.07101682
- Etkin A, Prater KE, Hoefl F, Menon V, Schatzberg AF. Failure of anterior cingulate activation and connectivity with the amygdala during implicit regulation of emotional processing in generalized anxiety disorder. *Am J Psychiatry.* (2010) 167:545–54. doi: 10.1176/appi.ajp.2009.09070931

8. Paulesu E, Sambugaro E, Torti T, Danelli L, Ferri F, Scialfa G, et al. Neural correlates of worry in generalized anxiety disorder and in normal controls: a functional MRI study. *Psychol Med.* (2010) 40:117–24. doi: 10.1017/S0033291709005649
9. Zugman A, Jett L, Antonacci C, Winkler AM, Pine DS. A systematic review and meta-analysis of resting-state fMRI in anxiety disorders: Need for data sharing to move the field forward. *J Anxiety Disord.* (2023) 99:102773. doi: 10.1016/j.janxdis.2023.102773
10. Madonna D, Delvecchio G, Soares JC, Brambilla P. Structural and functional neuroimaging studies in generalized anxiety disorder: a systematic review. *Braz J Psychiatry.* (2019) 41:336–62. doi: 10.1590/1516-4446-2018-0108
11. Makovac E, Meeten F, Watson DR, Herman A, Garfinkel SN, D Critchley H, et al. Alterations in amygdala-prefrontal functional connectivity account for excessive worry and autonomic dysregulation in generalized anxiety disorder. *Biol Psychiatry.* (2016) 80:786–95. doi: 10.1016/j.biopsych.2015.10.013
12. Kim N, Kim MJ. Altered task-evoked corticolimbic responsivity in generalized anxiety disorder. *Int J Mol Sci.* (2021) 22:3630. doi: 10.3390/ijms22073630
13. Szucs D, Ioannidis JP. Sample size evolution in neuroimaging research: An evaluation of highly-cited studies (1990–2012) and of latest practices (2017–2018) in high-impact journals. *Neuroimage.* (2020) 221:117164. doi: 10.1016/j.neuroimage.2020.117164
14. Elliott ML, Knodt AR, Ireland D, Morris ML, Poulton R, Ramrakha S, et al. What is the test-retest reliability of common task-functional MRI measures? New empirical evidence and a meta-analysis. *Psychol Sci.* (2020) 31:792–806. doi: 10.1177/0956797620916786
15. Infantolino ZP, Luking KR, Sauder CL, Curtin JJ, Hajcak G. Robust is not necessarily reliable: From within-subjects fMRI contrasts to between-subjects comparisons. *Neuroimage.* (2018) 173:146–52. doi: 10.1016/j.neuroimage.2018.02.024
16. Ma Z, Wang C, Hines CS, Lu X, Wu Y, Xu H, et al. Frontoparietal network abnormalities of gray matter volume and functional connectivity in patients with generalized anxiety disorder. *Psychiatry Res Neuroimaging.* (2019) 286:24–30. doi: 10.1016/j.pscychres.2019.03.001
17. Fan S, Yu Y, Wu Y, Kai Y, Wang H, Chen Y, et al. Altered brain entropy and functional connectivity patterns in generalized anxiety disorder patients. *J Affect Disord.* (2023) 332:168–75. doi: 10.1016/j.jad.2023.03.062
18. Chen Y, Cui Q, Sheng W, Tang Q, Lu F, Pang Y, et al. Anomalous neurovascular coupling in patients with generalized anxiety disorder evaluated by combining cerebral blood flow and functional connectivity strength. *Prog Neuropsychopharmacol Biol Psychiatry.* (2021) 111:110379. doi: 10.1016/j.pnpbp.2021.110379
19. Guo X, Yang F, Fan L, Gu Y, Ma J, Zhang J, et al. Disruption of functional and structural networks in first-episode, drug-naïve adolescents with generalized anxiety disorder. *J Affect Disord.* (2021) 284:229–37. doi: 10.1016/j.jad.2021.01.088
20. Jiang T, Yin X, Zhu L, Wang G, Zhang F, Guo J. Comparison of resting-state brain activity between insomnia and generalized anxiety disorder: A coordinate-based meta-analysis. *Brain Imaging Behav.* (2025) 19:218–39. doi: 10.1007/s11682-024-00949-9
21. Liao M, Yang F, Zhang Y, He Z, Su L, Li L. White matter abnormalities in adolescents with generalized anxiety disorder: a diffusion tensor imaging study. *BMC Psychiatry.* (2014) 14:41. doi: 10.1186/1471-244X-14-41
22. Ou CH, Cheng CS, Lin PL, Lee CL. Grey matter alterations in generalized anxiety disorder: A voxel-wise meta-analysis of voxel-based morphometry studies. *Int J Dev Neurosci.* (2024) 84:281–92. doi: 10.1002/ijdn.10330
23. Harrewijn A, Cardinale EM, Groenewold NA, Bas-Hoogendam JM, Aghajani M, Hilbert K, et al. Cortical and subcortical brain structure in generalized anxiety disorder: findings from 28 research sites in the ENIGMA-Anxiety Working Group. *Transl Psychiatry.* (2021) 11:502. doi: 10.1038/s41398-021-01622-1
24. Cui H, Zhang B, Li W, Li H, Pang J, Hu Q, et al. Insula shows abnormal task-evoked and resting-state activity in first-episode drug-naïve generalized anxiety disorder. *Depress Anxiety.* (2020) 37:632–44. doi: 10.1002/da.23009
25. Whitfield-Gabrieli S, Nieto-Castanon A. Conn: a functional connectivity toolbox for correlated and anticorrelated brain networks. *Brain Connect.* (2012) 2:125–41. doi: 10.1089/brain.2012.0073
26. Power JD, Mitra A, Laumann TO, Snyder AZ, Schlaggar BL, Petersen SE. Methods to detect, characterize, and remove motion artifact in resting state fMRI. *Neuroimage.* (2014) 84:320–41. doi: 10.1016/j.neuroimage.2013.08.048
27. Calhoun VD, Wager TD, Krishnan A, Rosch KS, Seymour KE, Nebel MB. The impact of T1 versus EPI spatial normalization templates for fMRI data analyses. *Hum Brain Mapp.* (2017) 38:5331–42. doi: 10.1002/hbm.23737
28. Hallquist MN, Hwang K, Luna B. The nuisance of nuisance regression: spectral misspecification in a common approach to resting-state fMRI preprocessing reintroduces noise and obscures functional connectivity. *Neuroimage.* (2013) 82:208–25. doi: 10.1016/j.neuroimage.2013.05.116
29. Nieto-Castanon A. Functional connectivity measures. In: *Handbook of functional connectivity Magnetic Resonance Imaging methods in CONN.* Boston, MA: Hilbert Press (2020). p. 26–62.
30. Desikan RS, Ségonne F, Fischl B, Quinn BT, Dickerson BC, Blacker D. An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. *Neuroimage.* (2006) 31:968–80. doi: 10.1016/j.neuroimage.2006.01.021
31. Nieto-Castanon A. General linear model. In: *Handbook of functional connectivity Magnetic Resonance Imaging methods in CONN.* Boston, MA: Hilbert Press (2020). p. 63–82.
32. Worsley KJ, Marrett S, Neelin P, Vandal AC, Friston KJ, Evans AC. A unified statistical approach for determining significant signals in images of cerebral activation. *Hum Brain Mapp.* (1996) 4:58–73. doi: 10.1002/(SICI)1097-0193(1996)4:1<58::AID-HBM4>3.0.CO;2-O
33. Chumbley J, Worsley K, Flandin G, Friston K. Topological FDR for neuroimaging. *Neuroimage.* (2010) 49:3057–64. doi: 10.1016/j.neuroimage.2009.10.090
34. Smith KA, Akhil Raja K, Arun KM, Rajesh PG, Thomas B, Kapilamoorthy TR, et al. Resting state fMRI: A review on methods in resting state connectivity analysis and resting state networks. *Neuroradiol J.* (2017) 30:305–17. doi: 10.1177/1971400917697342
35. Poldrack RA. Region of interest analysis for fMRI. *Soc Cognit Affect Neurosci.* (2007) 2:67–70. doi: 10.1093/scan/nsm006
36. Bullmore ET, Suckling J, Overmeyer S, Rabe-Hesketh S, Taylor E, Brammer MJ. Global, voxel, and cluster tests, by theory and permutation, for a difference between two groups of structural MR images of the brain. *IEEE Trans Med Imaging.* (1999) 18:32–42. doi: 10.1109/42.750253
37. Maknojia S, Churchill NW, Schweizer TA, Graham SJ. Resting state fMRI: going through the motions. *Front Neurosci.* (2019) 13:825. doi: 10.3389/fnins.2019.00825
38. Lopez C, Blanke O. The thalamocortical vestibular system in animals and humans. *Brain Res Rev.* (2011) 67:119–46. doi: 10.1016/j.brainresrev.2010.12.002
39. Yang F, Fan L, Zhai T, Lin Y, Wang Y, Ma J, et al. Decreased intrinsic functional connectivity in first-episode, drug-naïve adolescents with generalized anxiety disorder. *Front Hum Neurosci.* (2019) 12:539. doi: 10.3389/fnhum.2018.00539
40. Molent C, Maggioni E, Cecchetto F, Garzitto M, Piccin S, Bonivento C, et al. Reduced cortical thickness and increased gyrfication in generalized anxiety disorder: a 3 T MRI study. *Psychol Med.* (2018) 48:2001–10. doi: 10.1017/S003329171700352X
41. White T, Gottesman I. Brain connectivity and gyrfication as endophenotypes for schizophrenia: weight of the evidence. *Curr Top Med Chem.* (2012) 12:2393–403. doi: 10.2174/156802612805289953
42. Wada S, Honma M, Masaoka Y, Yoshida M, Koiwa N, Sugiyama H, et al. Volume of the right supramarginal gyrus is associated with a maintenance of emotion recognition ability. *PLoS One.* (2021) 16:e0254623. doi: 10.1371/journal.pone.0254623
43. Silani G, Lamm C, Ruff CC, Singer T. Right supramarginal gyrus is crucial to overcome emotional egocentricity bias in social judgments. *J Neurosci.* (2013) 33:15466–76. doi: 10.1523/JNEUROSCI.1488-13.2013
44. Esménio S, Soares JM, Oliveira-Silva P, Gonçalves ÓF, Decety J, Coutinho J. Brain circuits involved in understanding our own and other's internal states in the context of romantic relationships. *Soc Neurosci.* (2019) 14:729–38. doi: 10.1080/17470919.2019.1586758
45. Liégeois F, Mayes A, Morgan A. Neural correlates of developmental speech and language disorders: evidence from neuroimaging. *Curr Dev Disord Rep.* (2014) 1:215–27. doi: 10.1007/s40474-014-0019-1
46. Reynaud E, Lesourd M, Navarro J, Osiurak F. On the neurocognitive origins of human tool use: A critical review of neuroimaging data. *Neurosci Biobehav Rev.* (2016) 64:421–37. doi: 10.1016/j.neubiorev.2016.03.009
47. Andrews-Hanna JR, Reidler JS, Sepulcre J, Poulin R, Buckner RL. Functional-anatomic fractionation of the brain's default network. *Neuron.* (2010) 65:550–62. doi: 10.1016/j.neuron.2010.02.005
48. Li R, Shen F, Sun X, Zou T, Li L, Wang X, et al. Dissociable salience and default mode network modulation in generalized anxiety disorder: a connectome-wide association study. *Cereb Cortex.* (2023) 33:6354–65. doi: 10.1093/cercor/bhac509
49. Modi S, Kumar M, Kumar P, Khushu S. Aberrant functional connectivity of resting state networks associated with trait anxiety. *Psychiatry Res.* (2015) 234:25–34. doi: 10.1016/j.pscychres.2015.07.006
50. Pang X, Fan S, Zhang Y, Zhang T, Hou Q, Wu Y, et al. Alterations in neural circuit dynamics between the limbic network and prefrontal/default mode network in patients with generalized anxiety disorder. *NeuroImage Clin.* (2024) 43:103640. doi: 10.1016/j.nicl.2024.103640
51. Akiki TJ, Jubeir J, Bertrand C, Tozzi L, Williams LM. Neural circuit basis of pathological anxiety. *Nat Rev Neurosci.* (2025) 26:5–22. doi: 10.1038/s41583-024-00880-4
52. Camacho MC, Schwarzlose RF, Perino MT, Labonte AK, Koira S, Barch DM, et al. Youth generalized anxiety and brain activation states during socioemotional processing. *JAMA Psychiatry.* (2025) 82:264–73. doi: 10.1001/jamapsychiatry.2024.4105
53. Grillon C, Baas JM, Cornwell B, Johnson L. Context conditioning and behavioral avoidance in a virtual reality environment: effect of predictability. *Biol Psychiatry.* (2006) 60:752–9. doi: 10.1016/j.biopsych.2006.03.072
54. Shulman GL, Fiez JA, Corbetta M, Buckner RL, Miezin FM, Raichle ME, et al. Common blood flow changes across visual tasks: II. Decreases in cerebral cortex. *J Cognit Neurosci.* (1997) 9:648–63. doi: 10.1162/jocn.1997.9.5.648
55. Raichle ME, MacLeod AM, Snyder AZ, Powers WJ, Gusnard DA, Shulman GL. A default mode of brain function. *Proc Natl Acad Sci U.S.A.* (2001) 98:676–82. doi: 10.1073/pnas.98.2.676

56. Dosenbach NU, Fair DA, Cohen AL, Schlaggar BL, Petersen SE. A dual-networks architecture of top-down control. *Trends Cognit Sci.* (2008) 12:99–105. doi: 10.1016/j.tics.2008.01.001
57. Khalsa SS, Adolphs R, Cameron OG, Critchley HD, Davenport PW, Feinstein JS, et al. Interoception and mental health: A roadmap. *Biol Psychiatry Cognit Neurosci Neuroimaging.* (2018) 3:501–13. doi: 10.1016/j.bpsc.2017.12.004
58. Avery JA, Gotts SJ, Kerr KL, Burrows K, Ingeholm JE, Bodurka J, et al. Convergent gustatory and viscerosensory processing in the human dorsal mid-insula. *Hum Brain Mapp.* (2017) 38:2150–64. doi: 10.1002/hbm.23510
59. Critchley HD, Garfinkel SN. Interoception and emotion. *Curr Opin Psychol.* (2017) 17:7–14. doi: 10.1016/j.copsyc.2017.04.020
60. Fonzo GA, Etkin A. Affective neuroimaging in generalized anxiety disorder: an integrated review. *Dialogues Clin Neurosci.* (2017) 19:169–79. doi: 10.31887/DCNS.2017.19.2/gfonzo
61. Fitzgerald JM, Phan KL, Kennedy AE, Shankman SA, Langenecker SA, Klumpp H. Prefrontal and amygdala engagement during emotional reactivity and regulation in generalized anxiety disorder. *J Affect Disord.* (2017) 218:398–406. doi: 10.1016/j.jad.2017.05.013
62. Xu X, Dai J, Chen Y, Liu C, Xin F, Zhou X, et al. Intrinsic connectivity of the prefrontal cortex and striato-limbic system respectively differentiate major depressive from generalized anxiety disorder. *Neuropsychopharmacology.* (2021) 46:791–8. doi: 10.1038/s41386-020-00868-5
63. Liu S, Cao L, Li H, Du Y, Wang M, Xiao H, et al. Trait anxiety mediates the association between hippocampal-insula functional connectivity and anxiety symptom severity in adults with and without generalized anxiety disorder. *J Affect Disord.* (2024) 344:1–7. doi: 10.1016/j.jad.2023.10.006
64. Kolesar TA, Bilevicius E, Wilson AD, Kornelsen J. Systematic review and meta-analyses of neural structural and functional differences in generalized anxiety disorder and healthy controls using magnetic resonance imaging. *NeuroImage Clin.* (2019) 24:102016. doi: 10.1016/j.nicl.2019.102016
65. Chen Y, Cui Q, Xie A, Pang Y, Sheng W, Tang Q, et al. Abnormal dynamic functional connectivity density in patients with generalized anxiety disorder. *J Affect Disord.* (2020) 261:49–57. doi: 10.1016/j.jad.2019.09.084
66. Goodwin H, Yiend J, Hirsch CR. Generalized Anxiety Disorder, worry and attention to threat: A systematic review. *Clin Psychol Rev.* (2017) 54:107–22. doi: 10.1016/j.cpr.2017.03.006
67. Altemus M, Sarvaiya N, Neill Epperson C. Sex differences in anxiety and depression clinical perspectives. *Front Neuroendocrinol.* (2014) 35:320–30. doi: 10.1016/j.yfrne.2014.05.004
68. Bangasser DA, Cuarenta A. Sex differences in anxiety and depression: circuits and mechanisms. *Nat Rev Neurosci.* (2021) 22:674–84. doi: 10.1038/s41583-021-00513-0
69. Leichsenring F, Heim N, Steinert C. A review of anxiety disorders. *JAMA.* (2023) 329:1315–6. doi: 10.1001/jama.2023.2428
70. Yoo K, Lee P, Chung MK, Sohn WS, Chung SJ, Na DL, et al. Degree-based statistic and center persistency for brain connectivity analysis. *Hum Brain Mapp.* (2017) 38:165–81. doi: 10.1002/hbm.23352
71. Button KS, Ioannidis JP, Mokrysz C, Nosek BA, Flint J, Robinson ES, et al. Power failure: why small sample size undermines the reliability of neuroscience. *Nat Rev Neurosci.* (2013) 14:365–76. doi: 10.1038/nrn3475
72. Wang M, Cao L, Li H, Xiao H, Ma Y, Liu S, et al. Dysfunction of resting-state functional connectivity of amygdala subregions in drug-naïve patients with generalized anxiety disorder. *Front Psychiatry.* (2021) 12:758978. doi: 10.3389/fpsy.2021.758978
73. Cui H, Zhang J, Liu Y, Li Q, Li H, Zhang L, et al. Differential alterations of resting-state functional connectivity in generalized anxiety disorder and panic disorder. *Hum Brain Mapp.* (2016) 37:1459–73. doi: 10.1002/hbm.23113
74. Qiao J, Li A, Cao C, Wang Z, Sun J, Xu G. Aberrant functional network connectivity as a biomarker of generalized anxiety disorder. *Front Hum Neurosci.* (2017) 11:626. doi: 10.3389/fnhum.2017.00626
75. Domschke K, Stevens S, Pfeleiderer B, Gerlach AL. Interoceptive sensitivity in anxiety and anxiety disorders: an overview and integration of neurobiological findings. *Clin Psychol Rev.* (2010) 30:1–11. doi: 10.1016/j.cpr.2009.08.008
76. Chen WG, Schloesser D, Arensdorf AM, Simmons JM, Cui C, Valentino R, et al. The emerging science of interoception: sensing, integrating, interpreting, and regulating signals within the self. *Trends Neurosci.* (2021) 44:3–16. doi: 10.1016/j.tins.2020.10.007