Impact of self-designed Ningxin Anshen Decoction on the resting-state network functional connectivity in patients with mild to moderate generalized anxiety disorders

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Background: Generalized anxiety disorder (GAD) is a persistent chronic excessive anxiety that is hard to control. Our previous study indicated that self-designed Ningxin Anshen Formula (NXAS) was effective to treat mild to moderate GAD patients. This study is a randomized controlled clinical trial and aimed to investigate the imapct of self-designed NXAS on the resting-state functional connectivity (rsFC) in patients with mild to moderate GAD and explore the potential mechanisms.

Methods: A total of 61 patients diagnosed with mild to moderate GAD were recruited and divided into two groups randomly: NXAS group (n=31) and placebo group (n=30). Before and after treatment, the rsFC was examined by resting-state functional MRI (rs-fMRI), the anxiety was assessed with HAMA, and the independent component analysis (ICA) was used to analyze the resting-state functional connectivity (rsFC). The correlation between HAMA score and abnormal rsFC was further evaluated.

Results: The default mode network (DMN) showed evident rsFC interaction in the PCUN in both groups before and after therapy. The salience network (SN) showed obvious rsFC interaction in the bilateral gyrus frontalis inferiors and bilateral gyri temporalis superiors before and after therapy. In the NXAS group, the rsFC interction reduced significantly in the left gyrus frontalis inferior, but remained unchanged in the right gyrus frontalis inferior and bilateral gyri temporalis superiors after therapy. In the control group, the rsFC interaction increased dramatically after treatment. In addition, the abnormal rsFC had no relationship with HAMA score.

Conclusions: The self-designed NXAS can increase the rsFC in the PUCN on DMN and reduce rsFC in the orbIFG.L on SN to exert anti-anxiety effect.

Keywords: Generalized anxiety disorder (GAD); self-designed Ningxin Anshen Decoction; default mode network (DMN); salience network; resting-state functional connectivity (rsFC)

Introduction

Generalized anxiety disorder (GAD) is a persistent chronic excessive anxiety that is hard to control, and the common causes of GAD include the family, health, finance and other problems. Usually, GAD is accompanied by other non-specific psychological and physiological symptoms (1). The main characteristics of GAD are chronic excessive anxiety and worry (2), which significantly affects the work efficiency and increases the risk for other diseases. Among anxiety disorders, GAD has the highest prevalence and affects 4–6% of the general population (3). Treatment for GAD often includes medications such as selective serotonin reuptake inhibitors and/or psychotherapy, and cognitive behavior therapy has been studied widely among psychotherapeutic treatments. Benzodiazepines are also effective in reducing anxiety symptoms, but their use is limited by risk of abuse and adverse effect profiles. Physical activity can reduce symptoms of GAD. Moreover, a number of complementary and alternative treatments are often used (4). Traditional Chinese medicine (TCM) is a kind of complementary and alternative medicine and has unique theory and abundant preventative and therapeutic methods (5). Our previous study indicated that self-designed Ningxin Anshen Formula (NXAS) was effective to improve the symptoms and Hamilton anxiety scale (HAMA) of mild to moderate GAD patients with the overall effectiveness rate of 83.33%, which was significantly higher than in the placebo group (40%; P<0.01) (6).

The magnetic resonance imaging (MRI) can non-invasively assess the state of brain connectivity and determine whether it is dynamic (functional connectivity) or structural (structural connectivity). The resting-state functional MRI (rs-fMRI) can show the function and structure of the brain by assessing the signal changes in the brain blood oxygen at the baseline, which reflects the real time local activity of brain tissues (7). In recent years, rs-fMRI has been widely used to assess the brain dysfunction caused by various mental disorders. Some studies have shown that the resting-state whole brain functional networks are related to the anxiety (8,9).

The default mode network (DMN) is a group of functionally interconnected brain regions whose activity consistently decreases during the goal-oriented external tasks and/or increases during awake rest (10,11). The DMN is one of the resting state functional networks and contains meaningful information related to the spontaneous brain activity (12). It has been proposed that the DMN plays a key role in orchestrating the cognitive functions such as introspection, prospective memory, and a variety of processes described intuitively as daydreaming or mind-wandering (13,14). The DMN dysfunction has also been implicated in some pathological states including autism (15), schizophrenia (16), and depression (17).

In contrast to the DMN, the human SN is a distributed set of cortical and subcortical regions involved in detecting and responding to highly relevant (salient) stimuli (18,19). The SN was first identified using conventional fMRI to measure the blood oxygen level-dependent (BOLD) responses during a spatial working memory task in patients (18). In this task, several cortical regions (including the frontoinsular cortex) display significant BOLD responses. The insular cortex is subsequently used as a "seed region" for an independent component analysis of its functional connectivity patterns. The known functions and modalities processed in these brain regions include attention, sensation, visceral activity, affection, limbic system) and thus investigators speculate that this network processes perceptual salience and thus term it the "salience network" (20). The discovery of the SN also reveals an interesting clinical relationship: the strength of functional connectivity within the SN is strongly correlated with visual analog scores from a pre-scan anxiety assessment. This correlation suggests a tight link between the strength of the SN and states of vigilance (20). These findings are also confirmed by recent studies. In addition, studies also indicate that functional connectivity within the SN is aberrant in many neurological disorders including anxiety (21), post-traumatic stress disorder (22), depression (19), psychosis with auditory delusions (23), and affective disorders (24). In our previous study (6), results showed self-designed Ningxin Anshen (NXAS) Decoction was effective to improve the clinical symptoms of mild to moderate GAD, but the specific mechanism is still unclear. In recent years, great progress has been achieved in the use of rs-fMRI in the neuropsychiatric disorders. We hypothesized that Ningxin Anshen Formula could relieve GAD by improving the rsFC of GAD. In the present study, rs-fMRI was performed before and after treatment with NXAS Decoction in patients with mild to moderate GAD, aiming to explore the potential mechanism underlying the therapeutic effect of NXAS Decoction on the GAD.

We present the following article in accordance with the CONSORT reporting checklist (available at http://dx.doi.org/10.21037/apm-20-300).

Methods

Our clinical trial is a double-blind, randomized, parallel controlled trial and we have not made any significant changes to the test method since the start of the test (e.g., qualification criteria). Because we hypothesized that Ningxin Anshen Formula could relieve GAD by improving the rsFC of GAD. We want to observe the changes of rsFC before and after NXAS Decoction treatment of GAD, and prove it.

Participants

The sample size was estimated from a previous study that evaluated GAD differences between two groups as its primary outcome. The effective rate of NXAS group was $\pi1$, and that of placebo group was $\pi2$. According to the results of previous research, $\pi1\approx87$, $\pi2\approx47$, $f(\alpha,\beta)=10.5(\alpha=0.05$, $\beta=0.1)$ (5).

$$n1 = n2 = \frac{n1(100-\pi1)+\pi2(100-\pi2)}{(\pi1-\pi2)^2}f(\alpha,\beta)$$

As a result, n1or n2 is at least 24 and considering the loss rate of at least 20%. A total of 72 GAD patients were recruited from the Department of Medical Neurology at Tongzhou zone of Dongzhimen Hospital of Beijing University of Chinese Medicine between November 2015 and June 2017. A researcher who was not involved in the treatment and data analysis handed out the serial number and opaque, sealed envelopes to patients. They were randomly allocated to receive the experimental group (NXAS group, Group A) or control group (Placebo group, Group B) based on the odd (Group A) or even (Group B) numbers assigned in the envelope. All patients in both groups received treatment for 4 weeks. In order to maintain blinding through this clinical trial, the patients, statisticians, doctors and the data evaluators were blind to the allocation of the groups. During the intervention period, doctors and data collection staff would visit patients at different time intervals to avoid exchange of information. Only by Unblinding we only know the result after the end of the trial. The GAD was diagnosed by two experienced psychiatrists. GAD patients were randomized double-blindly into NXAS group and placebo group (n=36 per group). During the study period, 1 patient was excluded and 4 were lost to follow up in the NXAS group; in the placebo group, 1 was excluded, and five were lost to follow up. Of 9 patients who were lost to follow up, 5 did not receive medication one time, 3 refused to continue treatment and

1 was not in contact with the physicians. In addition, 2 patients received treatment with other anti-anxiety drugs and thus excluded from this study. These patients were not included in final analysis. The inclusion criteria were as follows: (I) the patients were diagnosed with GAD according to the diagnostic criteria from the American Diagnostic and Statistical Manual of Mental Disorders (5th ed) (25) and not treated before admission; (II) patients complained of uncontrollable anxiety and worried about everyday events and problems for at least 6 months; (III) patients were 20–40 years old; (IV) all the participants were right-handed; (V) GAD patients had the HAMA score higher than 14, but lower than 29 (HAMA score <21, mild; 29> HAMA score ≥21: moderate), the Hamilton Depression Scale (HAMD) score (17 items) was <7; (VI) patients were not diagnosed with mental retardation and had no history of head injury or neurological trauma that resulted in a loss of consciousness (26). The study protocol was approved by the Ethics Committee of Dongzhimen Hospital of Tongzhou District, Beijing University of Chinese Medicine Medical (NO. DZMEC-KY-201249) and registered on the Clinical Trials (NO. ChiCTR-OCC-12002329). Written informed consent was obtained from the patient for publication of this study and any accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal. And the study conformed to the provisions of the Declaration of Helsinki (as revised in 2013).

Exclusion criteria were as follows: (I) patients had a history of neurological or psychiatric disorders or cognitive dysfunction, such as depression, obsessive-compulsive disorder, schizophrenia, PTSD and bipolar disorder; (II) patients had severe liver, heart and kidney dysfunction; (III) patients were pregnant or breast-feeding women; (IV) patients also had secondary anxiety caused by hyperthyroidism and no history of alcohol or other substance abuse in the year before testing; (V) patients received other anti-depression or anti-anxiety drugs or hypnagogue; (VI) patients developed severe diseases that did not allow them to participate in this study.

Drugs

The investigated product was NXAS Decoction, a Chinese herbal medicine product composed of Suan Zao Ren (equivalent to 60 g of crude drug/package), Ci Wu Jia (equivalent to 45 g of crude drug/package), and Xia Ku Cao (equivalent to 10 g of crude drug/package). NXAS Decoction was extracted from these three kinds of crude

Table 1 General characteristics of patients in two groups ($\bar{x}\pm s$)

Group	n	Male	Female	Age (years)	Duration (months)	HAMA scores
NXAS	31	15	16	32±5.64	8.19±1.76	18.13±2.49
Placebo	30	13	17	30.87±5.8	8.47±2.13	17.87±2.43

traditional Chinese medicine and made into granule mixture in Beijing Kangrentang Pharmaceutical Co., Ltd. The placebo, composed of dextrin, had the same appearance to NXAS Decoction. The participants in the NXAS group and placebo group were orally treated with 1 bag of NXAS Formula and placebo, respectively, twice daily (one in the morning and one in the evening) for 4 weeks. If necessary, psychological counselling was administered to avoid emotional fluctuation. The general characteristics, the severity of anxiety and the course of disease are shown in *Table 1*.

Image acquisition

All participants were informed of the safety and eligibility criteria for fMRI scanning. rs-fMRI and structural data were acquired on a 1.5 Tesla MRI scanner (Philips Intera, Philips Medical Systems, Best, the Netherlands) with head coils in the Dongzhimen Hospital, Beijing University of Traditional Chinese Medicine. Patients received clinical assessments and rs-fMRI before and after 4-week treatment. Each subject lied on a foam pad to reduce head motion in a supine position with a headphone to reduce noise. In the data acquisition, all subjects were asked to close their eyes, relax, keep calm, not think about anything, and not fall asleep.

Rs-fMRI data were acquired using a single-shot gradient-echo planar imaging sequence, and the following parameters were used: echo time (TE) =50 ms, repetition time (TR) =3,000 ms, flip angle (FA) =90°, matrix =64×64, number of excitations = 1, slice thickness =3.6 mm, slice gap =0.72 mm, field of view (FOV) = 232 mm × 232 mm, voxel size = 3.63 mm × 3.68 mm × 3.6 mm, dynamics scans = 60. The rs-fMRI scanning in each subject lasted for 6 min and 12 sec. For the T1-weighted structural imaging, a two-dimensional inversion recovery turbo spin echo sequence was used, and the parameters were as follows: TE =15 ms, TR =5,165 ms, matrix = 384 × 384 × 75%, FA =90°, number of slices =33, FOV =232 mm × 232 mm, voxel size =0.6 mm × 0.82 mm × 3.6 mm, total duration =3.05 min. After an MRI scanning, the patient was assessed if he or she had followed the

instructions and remained awake throughout the scanning. Those who did not comply with the instructions were excluded from this study.

Image processing and independent component analysis

Rs-fMRI was performed in the State Key Laboratory of Cognitive Neuroscience and Learning of Beijing Normal University. First, all rs-fMRI data were subjected to pre-processing with DPABI software (Data Processing and Analysis of Brain Imaging; http://www.rfmri.org) (27). The format of raw data was transformed into NIFTI (.hdr/.img) format, 10 initial time points were removed aiming to avoid the influence of scanning noise and patients' inadaptability, and the remaining time points were used for further analysis. Then, Images were realigned for motion correction and slice timing correction. The images with the excessive head motion (>3 mm in shift or 3° in rotation) were removed. Thereafter, fMRI images were standardized to the Montreal Institute of Neurology template (resampling voxel size =3×3×3 mm^3). Linear trends and temporal filtering (band pass, 0.01–0.08 Hz) were removed to reduce very low frequency drift and physiologic high frequency respiratory and cardiac noise. Then, spatial smoothing was performed on the standardized individual with a Gaussian kernel of 6-mm full-width at half-maximum (28).

ICA was carried out for the analysis of fMRI data using the fMRI toolbox (GIFT; Group ICA for fMRI, http://icatb.sourceforge.net/) (29). Data from individual subjects were concatenated across time, and calculated for subject-specific components using the Infomax algorithm which is based on the principle of maximum information transfer (30). The Independent Components Number was 20. Best-fit DMN and SN components were selected manually using the template in the GIFT. Scans with problems related to best-fit DMN and SN component selection were excluded.

Statistical analysis

The DMN and SN at two time points were compared between two groups with independent sample *t*-test,

and FDR correction was done. The brain regions with significant difference were used for masking. The mask of each network in each group was made, and then the combination of two groups was taken as the statistical range of the network. Then, the Mixed effect Analysis was employed to analyze the interaction between group and time. The Permutation test for Threshold-Free Cluster Enhancement (TFCE) was employed for multiple comparisons and corrections (22), and significant brain regions with family-wise error (FEW) correction were obtained. Finally, the mean functional connectivity of each region was compared with repeated analysis of variance (repeated ANOVA), followed by Post hoc test.

The Fisher's r-to-z transformation was used to convert the correlation coefficient (r) into z-score so that the correlation coefficient would be distributed approximately normally. Finally, the functional connectivity of the DMN and SN was analyzed between 2 groups, and the z-scores of different regions were obtained. The Pearson's correlation analysis with 95% confidence interval (CI) was used to assess the correlation between HAMA scores and z-scores, and to evaluate the correlation between HAMA score and abnormal functional connectivity.

Results

Demographic and clinical characteristics

The GAD patients were randomized double-blindly into NXAS group and placebo group (n=36 per group). During the study period (between November 2015 and June 2017), 1 patient was excluded and 4 were lost to follow up in the NXAS group; in the placebo group, 1 was excluded, and five were lost to follow up. Of 9 patients who were lost to follow up, 5 did not receive medication one time, 3 refused to continue treatment and 1 was not in contact with the physicians. In addition, 2 patients received treatment with other anti-anxiety drugs and thus excluded from this study. These patients were not included in final analysis. There were 31 in NXAS group and 30 in placebo group at last. The trial is progressing smoothly and completed after 4-week treatment and there was no adverse event in both groups. For both groups, 61 participants included in each analysis and the analysis was by original assigned groups. As shown in *Table 1*, there were no significant differences in the gender (P=0.90), age (P=0.44) and course of disease (P=0.59) between two groups. The HAMA scores (P=0.68) before treatment was also similar between two groups.

Networks extracted by ICA

The DMN and salient SN extracted by ICA are shown in *Figure 1*. DMN mainly included bilateral medial prefrontal lobes, posterior cingulate/anterior cuneate lobe, angular gyrus and other brain regions. The SN mainly included the anterior cingulate gyrus, anterior insula and other brain regions.

Connectivity analysis in DMN

There was significant interaction in the PCUN (TFCE based FEW correction; P<0.05; *Table 2* and *Figure 2*). The means of this region were calculated and subjected to repeated-ANOVA for region of interest (ROI) analysis. As shown in *Figure 2* and *Table 3*, there was evident functional connectivity interaction in the PCUN (F=67.430, P<0.001), and the main effect of group (F=9.100, P=0.004) and main effect of time (F=8.764, P=0.004) were also significant. Post-hoc test showed the functional connectivity in the NXAS group (Group A) was significantly lower than in the placebo group (Group B) before treatment (P<0.001); after treatment, the functional connectivity in the NXAS group increased markedly (P=0.003), but it in the placebo group reduced dramatically (P<0.001). After 4-week treatment, the functional connectivity in the NXAS group was still significantly higher than in the placebo group (P=0.048) (*Figure 2* and *Table 4*). Self-designed NXAS decoction may treat the GAD by increasing the rsFC of PUCN.

Connectivity analysis in SN

The mixed effect analysis showed the significant interaction in the bilateral gyrus frontalis inferiors and gyri temporalis superiors (TFCE based FEW correction; P<0.05) (*Table 2* and *Figure 3*). The brain regions with significant interaction were divided into 4 clusters: left orbital gyrus frontalis inferior (orbIFG.L), right triangularis gyrus frontalis inferior (triIFG.R), left gyri temporalis superior (STG.L) and right gyri temporalis superior (STG.R).

The means of these regions were calculated and subjected to repeat-ANOVA for ROI analysis. As shown in *Figure 3* and *Table 3*, there was significant interaction in these four regions (orbIFG.L: F=43.543, P<0.001; STG.R: F=118.580, P<0.001; STG.L: F=107.195, P<0.001; triIFG.R: F=30.490, P<0.001). The significant main effect of group was noted in the orbIFG.L (F=10.266, P=0.002), STG.R (F=19.336, P<0.001) and STG.L (F=16.074, P<0.001), but not triIFG.
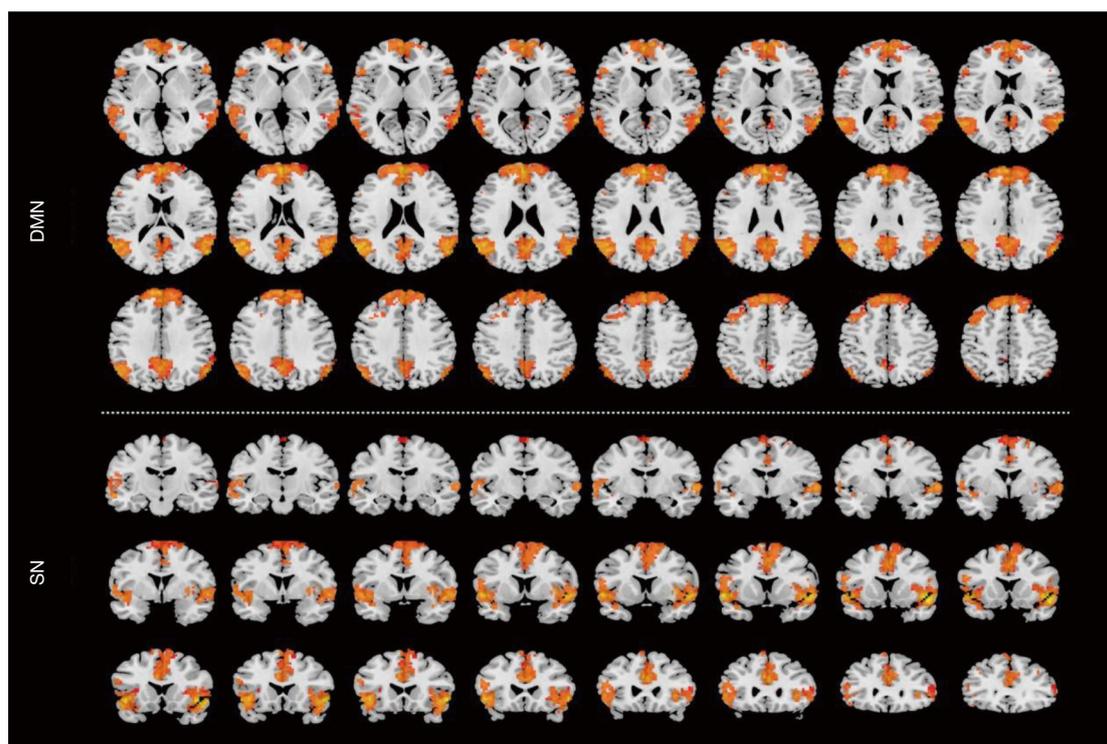
Figure 1 DMN and SN. Voxel intensities represent the connectivity and degree of coactivation in the resting state network. Voxels which were negatively associated with the network were excluded. DMN, default mode network; SN, salience network.

Table 2 Interaction in the PCUN

No	Region	Full name	F	Matrix	MNI		
					x	y	z
DMN							
1	PCUN	Precuneus (bilateral)	−4.1617	137	−9	−63	18
SN							
1	orbIFG.L	Left Inferior Frontal Gyrus (orbital part)	4.6971	104	−51	27	−3
2	STG.R	Right Superior Temporal Gyrus	6.9494	675	60	6	−6
3	STG.L	Left Superior Temporal Gyrus	5.8197	701	−60	−6	3
4	triIFG.R	Right Inferior Frontal Gyrus (triangular part)	4.5696	83	48	21	21

DMN, default mode network; SN, salience network.

R (F=0.031, P=0.861). The significant main effect of time was found in the STG.R (F=80.131, P<0.001), STG.L (F=99.888, P<0.001) and triIFG.R (F=32.156, P<0.001), but not orbIFG.L (F=3.069, P=0.085).

The post-hoc analysis showed the functional connectivity in four regions of NXAS group (Group A) was significantly higher than that of placebo group (Group B) (all P<0.001).

After treatment, the functional connectivity reduced significantly in the orbIFG.L, but it in remaining regions remained unchanged (STG.R: P=0.135; STG.L: P=0.807; triIFG.R: P=0.913) in the NXAS group. In the placebo group, the functional connectivity of four regions increased markedly after treatment (all P<0.001). In addition, after treatment, the functional connection in the STG.R, STG.
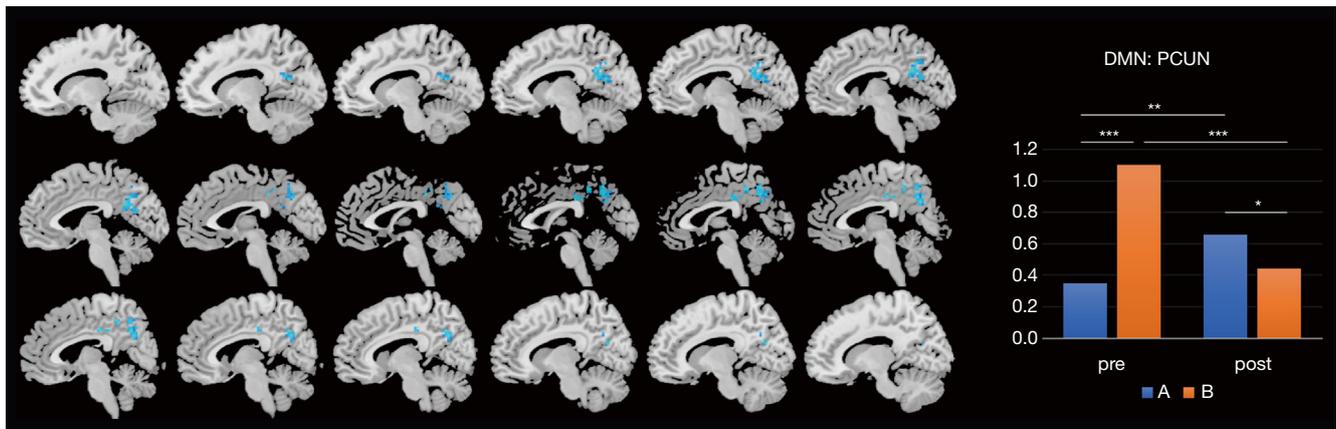
Figure 2 rsFC in DMN before and after treatment in the NXAS group and placebo group. rsFC, resting-state functional connectivity; DMN, default mode network; NXAS, ningxin Anshen Formula.

Table 3 Repeat-ANOVA of ROI

No	Region	Functional connectivity (mean)				Interaction		Main effect of group		Main effect of time	
		A-pre	A-post	B-pre	B-post	F	P	F	P	F	P
DMN											
1	PCUN	0.346	0.657	1.101	0.441	67.430	<0.001	9.100	0.004	8.764	0.004
SN											
1	orbIFG.L	0.973	0.566	0.025	0.726	43.543	<0.001	10.266	0.002	3.069	0.085
2	STG.R	0.676	0.582	−0.104	0.857	118.580	<0.001	19.336	<0.001	80.131	<0.001
3	STG.L	0.537	0.520	−0.166	0.798	107.195	<0.001	16.074	<0.001	99.888	<0.001
4	triIFG.R	0.260	0.275	−0.330	0.826	30.490	<0.001	0.031	0.861	32.156	<0.001

ROI, region of interest; DMN, default mode network; SN, salience network.

Table 4 *Post-hoc* analysis of ROI

No.	Regions	Pre-post		A-B	
		A	B	Pre	Post
DMN					
1	PCUN	0.003	<0.001	<0.001	0.048
SN					
1	orbIFG.L	0.002	<0.001	<0.001	0.273
2	STG.R	0.135	<0.001	<0.001	0.001
3	STG.L	0.807	<0.001	<0.001	<0.001
4	triIFG.R	0.913	<0.001	<0.001	<0.001

DMN, default mode network; SN, salience network.

L and triIFG.R of NXAS group was markedly lower than in the placebo group (P=0.001 or <0.001), but it in the orbIFG.L was similar between two groups (P=0.273) (*Figure 3* and *Table 4*). Self-designed NXAS decoction may relieve the symptoms of GAD in decreasing the rsFC of orbIFG.L.

Correlation between functional connectivity and GAD severity

Results showed the extracted functional connectivity of DMN and SN in GAD patients had no relationship with the HAMA scores at baseline. This result might be ascribed to the small size of sample.
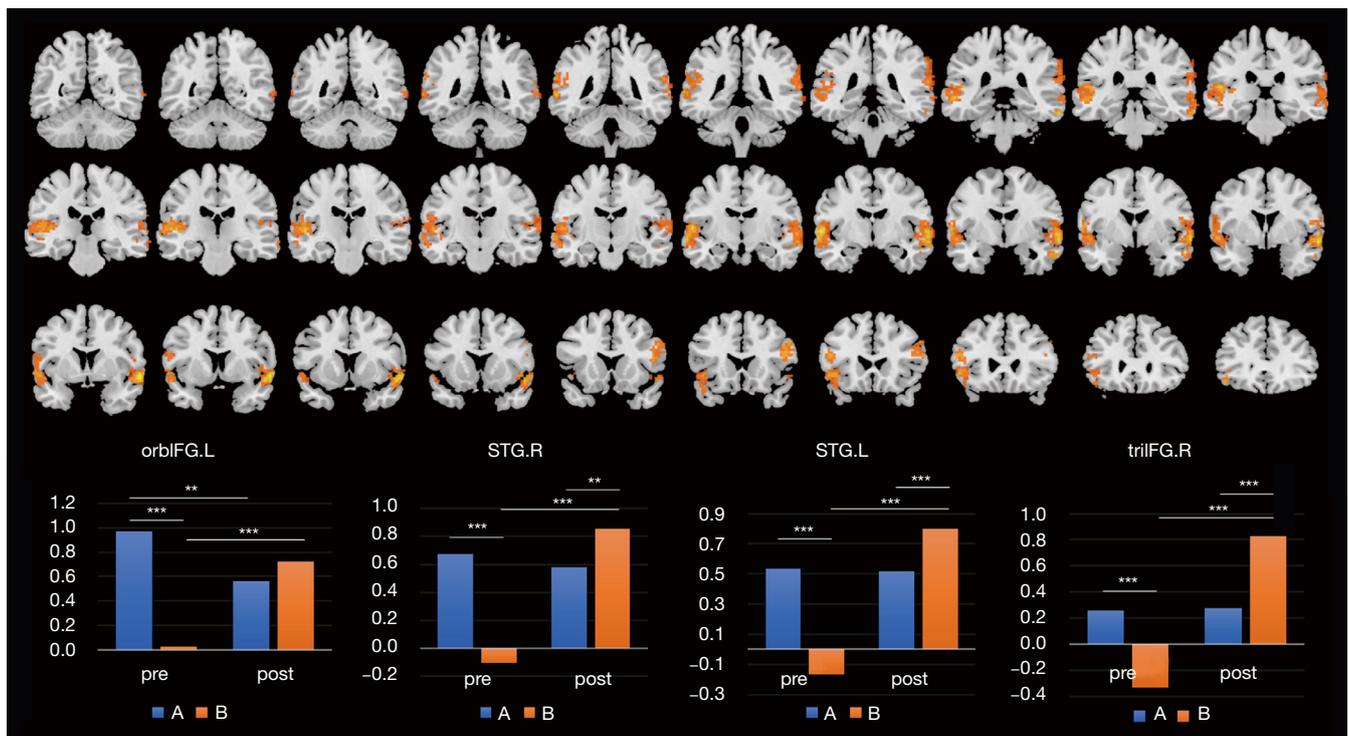
Figure 3 rsFC in SN before and after treatment in the NXAS group and placebo group. rsFC, resting-state functional connectivity; SN, salience network; NXAS, ningxin Anshen Formula.

Discussion

Although rs-fMRI has been used more extensively in many studies about psychiatric disorders owing to the relative ease of acquiring data with this system, few studies have been conducted to investigate GAD by using rs-fMRI. In the present study, the rsFC was assessed by rs-fMRI in patients with mild to moderate GAD before and after treatment with NXAS decoction, and the potential mechanism underlying the anti-anxiety of NXAS decoction was explored.

A variety of methods have been employed in the studies about rs-fMRI. In contrast to seed-based analysis, which heavily depends on a priori seeds defined, ICA requires no prior knowledge of a network, and hence may provide complementary/confirmatory information (31). ICA by definition is a blind source deconvolution technique which is considered an optimal method for separating signals from different sources (32). Thus, ICA was employed for the analysis of brain functional connectivity networks in the present study.

Major connectivity networks such as the DMN and SN can have aberrant connectivity patterns that are thought to underlie a host of neurological and psychiatric disorders (19,33). The DMN is a major functional brain system which has been implicated in a number of psychiatric disorders (34). The DMN is important for the social understanding of others (35), being engaged in tasks involving evaluative social processes (34,36), mentalizing and theory of mind (14,37). Studies have shown the involvement of DMN in the processing of acute stress induction without on-going task demands that was not captured in the previous investigations (38,39). The DMN has largely been linked to self-referential processes (14,40). Alterations in the DMN connectivity have consistently been implicated in various psychiatric disorders and particularly in stress-related disorders. For example, reduced baseline DMN connectivity is related to PTSD in patients. Similarly, our study also revealed that the rsFC reduced in the DMN of GAD patients, and self-designed NXAS decoction was able to increase the rsFC, which may be one of mechanisms underlying the anti-anxiety effect of NXAS decoction.

The salience network is involved in orienting attention to external and internal stimuli, and facilitates the integration of sensory, emotional, and cognitive information in service of optimal communication, social

behavior, and self-awareness. When examined via functional neuroimaging, patients with GAD frequently exhibit SN abnormalities. In task-based studies, GAD patients consistently show hyperactivity of the anterior insula (41). Studies also reveal a strong correlation between anxiety severity and connectional strength of the SN, suggesting a role in anxiety disorders and pathological states of hyper vigilance (18,21). The amygdala has reciprocal connections with association cortices in the superior temporal gyri through which it can selectively modulate sensory responses depending on their emotional relevance (42). Our study also confirmed that, in the SN, there was significant interaction in bilateral IFG and bilateral STG of GAD patients. Although the rsFC of these regions in the NXAS group was significantly higher than in the placebo group before treatment, the rsFC of orbIFG.L in the NXAS group reduced significantly, but it in remaining regions remained unchanged after treatment. This indicates that NXAS decoction may reduce the rsFC of orbIFG.L to exert anti-anxiety effect, and the insignificant change in the remaining regions might be ascribed to the short course of treatment. In addition, our results showed the rsFC of these four regions after placebo treatment for 4 weeks increased significantly. Other studies also identified the increased connectivity in the SN of participants with high cortisol stress responses. SN has been implicated in diverse functions at the intersection of cognition and emotion including pain and negative affect processing (43) and integrating information relevant for cognitive control (44), suggesting potential involvement of this region in responding to challenging conditions and the appraisal and expression of anxiety (45).

rsFC studies demonstrated abnormalities and mostly decreases in DMN in GAD. In contrast, resting-state fMRI showed increased rsFC in SN of GAD. Since rsFC is coherence- or phase-based operating in the infraslow frequency domain (0.01–0.1 Hz), suggesting spatiotemporal hypo- or hyper-synchronization in DMN and SN, respectively. These abnormalities in the neural network's spatiotemporal synchronization may impact the phase-based temporal synchronization of neural and cardiac activities resulting in decreased (DMN) or increased (SN) neuro-cardiac coupling in GAD, which may be related to the various psychopathological symptoms like unstable sense of self (as based on unstable DMN showing spatiotemporal hypo-synchronization), increased emotions, and specifically anxiety (as related to increased SN showing spatiotemporal hyper-synchronization) in GAD. Taken together, we here

suggest that altered spatiotemporal synchronization of neural and cardiac activity within the brain's resting state underlies various psychopathological symptoms in anxiety disorders. Such spatiotemporal basis of psychopathological symptoms is well compatible with the recently suggested "Spatiotemporal Psychopathology" (46). And this study also revealed that the rsFC reduced in the DMN and increased in the SN of GAD patients, and self-designed NXAS decoction was able to increase the rsFC of PUCN and reduce the rsFC of orbIFG.L to relieve GAD, which may be one of the mechanisms underlying the anti-anxiety effect of NXAS decoction. Available findings are consistent with the idea of a stress-induced network reorganization and suggest that increased SN connectivity and decreased DMN connectivity may function as relevant neural indicators for stress responsiveness (47). In a more recent study (48), the results showed that both transcranial direct current stimulation (tDCS) and pharmacotherapy are effective in reducing worry symptoms, with similar efficacy between two treatments. Thus, the tDCS is a promising treatment for generalized anxiety disorder. In addition, in the absence of task, the main effect of tDCS is to accentuate DMN activation and SN deactivation (49). This was similar to the anti-anxiety effect of self-designed NXAS decoction in the present study.

Our previous study (6) showed the self-designed NXAS decoction was able to improve the clinical symptoms of mild to moderate GAD. In the present study, results showed no relationship between the functional connectivity networks and HAMA scores. Other study also shows no relationship between functional connectivity and depressive symptoms or Ruminative Response Scale scores in patients with severe depression (50). This might be related to the small sample size and exclusion of severe GAD patients. Thus, more studies with large sample size are needed to confirm our findings and to further assess the relationship between HAMA score and functional connectivity in GAD patients.

Limitations

There are several limitations in the present study. In particular, this was a preliminary study and NXAS was not compared with the widely used anti-anxiety drugs. Thus, the interpretation of our findings should be cautious. There was significant difference in the rsFC before treatment between two groups, which might be ascribed to the small sample size. However, the intragroup changes were significant after NXAS treatment, suggesting that NXAS may exert anti-

anxiety effect via improving rsFC. In addition, there were no healthy controls in our study and the sample size was small. Given the interest in investigating multiple nodes within the DMN and SN with several clinical variables of interest, larger sample size is needed in future studies to test the associations between these variables, as well as to assess confounding factors such as gender. Moreover, more studies are needed to elucidate the potential mechanisms underlying the anti-anxiety effect of self-designed NXAS. Despite the aforementioned limitations, the present findings still provide objective evidence on the change of functional connectivity in GAD patients.

Conclusions

GAD patients exhibit abnormalities in two brain networks (SN and DMN), reflecting self-referentiality. NXAS decoction is able to increase the rsFC of PUCN in DMN and reduce the rsFC of orbIFG.L in SN, exerting anti-anxiety effect.

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Footnote

Reporting Checklist: The authors have completed the CONSORT reporting checklist. Available at http://dx.doi.org/10.21037/apm-20-300

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aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study protocol was approved by the Ethics Committee of Dongzhimen Hospital of Tongzhou District, Beijing University of Chinese Medicine Medical (No. DZMEC-KY-201249) and registered on the Clinical Trials (No. ChiCTR-OCC-12002329). Written informed consent was obtained from the patient for publication of this study and any accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal. And the study conformed to the provisions of the Declaration of Helsinki (as revised in 2013).

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