Increased Local Spontaneous Neural Activity in the Left Precuneus Specific to Auditory Verbal Hallucinations of Schizophrenia

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Background: Auditory verbal hallucinations (AVHs) of schizophrenia have been associated with structural and functional alterations of some brain regions. However, the brain regional homogeneity (ReHo) alterations specific to AVHs of schizophrenia remain unclear. In the current study, we aimed to investigate ReHo alterations specific to schizophrenic AVHs.

Methods: Thirty-five schizophrenic patients with AVH, 41 schizophrenic patients without AVHs, and fifty healthy subjects underwent resting-state functional magnetic resonance imaging. ReHo differences across the three groups were tested using a voxel-wise analysis.

Results: Compared with the healthy control group, the two schizophrenia groups showed significantly increased ReHo in the right caudate and inferior temporal gyrus and decreased ReHo in the bilateral postcentral gyrus and thalamus and the right inferior occipital gyrus (false discovery rate corrected, \( P < 0.05 \)). More importantly, the AVH group exhibited significantly increased ReHo in the left precuneus compared with the non-AVH group. However, using correlation analysis, we did not find any correlation between the auditory hallucination rating scale score and the ReHo of brain regions.

Conclusions: Our results suggest that increased ReHo in the left precuneus may be a pathological feature exclusive to schizophrenic AVHs.

Key words: Auditory Verbal Hallucination; Default Mode Network; Precuneus; Regional Homogeneity; Schizophrenia

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Introduction

Auditory verbal hallucinations (AVHs) are among the core symptoms used in the diagnosis of schizophrenia, occurring in 60–80% of schizophrenia patients.1 Characterizing the brain structural and functional features of specific symptoms can enhance our understanding of the etiology of schizophrenia2 and provide objective indicators for precision medicine.3,4 Some neuroimaging data regarding the AVHs of schizophrenia have been obtained from structural magnetic resonance imaging (MRI) and functional MRI (fMRI) studies.5,6 Structural imaging studies have found that schizophrenia patients with AVHs usually exhibit gray matter volume reductions in the superior temporal gyrus.5-8 Complementary diffusion tensor imaging studies have found that the AVHs of schizophrenia are associated with disruptions of white matter integrity in the left arcuate fasciculus bundle and interhemispheric auditory fiber bundles.9,10 fMRI studies have found that the AVHs of schizophrenia are associated with abnormal activation within the default mode network (DMN) and auditory- and visual-associated resting-state (RS) networks.11-15 All the aforementioned findings advanced our understanding of the pathological traits of schizophrenic AVHs to some extent. However, considering the high heterogeneity and complexity of AVHs, for a comprehensive understanding of AVHs, we...
must use multiple techniques to characterize the pathological features of AVHs from different angles.

Regional homogeneity (ReHo)\[16\] measures the similarity of the time series of blood-oxygen-level-dependent (BOLD) signals of a given voxel to those of its nearest neighbors in a voxel-wise way, which reflects the local synchronization in the functional cluster. A specific region in the brain that is abnormal can be identified by ReHo; aberrant ReHo represents aberrant regional brain spontaneous activity in a specific region\[16,17\]. In recent years, ReHo has been used to investigate functional modulations in the RS of patients with schizophrenia and other mental disorders.\[18,19\]

In the current study, we were interested in whether schizophrenic patients with AVHs would demonstrate specific ReHo alterations and, if so, whether the brain regions (RS networks) with abnormal ReHo were specific to AVHs.

### Methods

#### Subjects

A total of 126 right-handed individuals were enrolled in the present study, including 76 schizophrenia patients and fifty healthy controls. The diagnosis of schizophrenia was determined by the consensus of two professional psychiatrists using the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders, 4th edition (SCID).\[20\] All healthy controls were screened using the nonpatient edition of the SCID to confirm a lifetime absence of psychiatric illnesses. Exclusion criteria for all subjects were a history of head trauma with consciousness disturbances lasting more than 5 min, a history of drug or alcohol abuse, pregnancy, and any physical illnesses, such as cardiovascular disease or neurological disorders, as diagnosed by an interview and medical records review. In addition, all healthy controls were interviewed to exclude individuals with a known history of psychiatric illness in first-degree relatives. Schizophrenia patients were subdivided into two groups according to their experience of AVHs. AVH group (n = 35) included patients who experienced AVHs at least once daily, and the non-auditory verbal hallucinations (nAVH) group (n = 41) included patients who had never experienced AVHs or had not experienced AVHs within 12 months before MRI. Clinical symptoms of psychosis were quantified using the positive and negative syndrome scale (PANSS).\[21\] The auditory hallucination rating scale\[22\] was used to assess AVH with seven characteristics such as frequency, reality, loudness, number of voices, length, attention dedicated to the hallucinations, and hallucination-induced arousal. All the patients were chronic schizophrenia patients and were receiving antipsychotic medication. The daily antipsychotic dosages (chlorpromazine equivalents) for two schizophrenia patient groups are listed in Table 1. The Medical Research Ethics Committee of Tianjin Medical University General Hospital approved this study. After receiving a complete description of the study, each subject provided written informed consent.

#### Data acquisition

MRI data were acquired using a 3.0-tesla MR system (Discovery MR750, General Electric, Milwaukee, WI, USA). Tight but comfortable foam padding was used to minimize head motion, and earplugs were used to reduce scanner noise. Sagittal three-dimensional T1-weighted images were acquired using a brain volume sequence with the following parameters: repetition time (TR) = 8.2 ms; echo time (TE) = 3.2 ms; inversion time = 450 ms; flip angle (FA) = 12°; field of view (FOV) = 256 mm × 256 mm; matrix = 256 × 256; slice thickness = 1 mm, no gap; 188 sagittal slices; and acquisition time = 250 s. RS functional BOLD images were acquired using a gradient-echo single-shot echo planar imaging sequence with the following parameters: TR/TE = 2000/45 ms, FOV = 220 mm × 220 mm, matrix = 64 × 64, FA = 90°, slice thickness = 4 mm, gap = 0.5 mm, 32 interleaved transverse slices, 180 volumes, and acquisition time = 370 s. All subjects were instructed to keep their eyes closed, relax, move as little as possible, think of nothing in particular, and not fall asleep during the scans. All MRI were visually inspected to ensure that only images without visible artifacts were included in subsequent analyses.

#### Data preprocessing

RS BOLD data were preprocessed using SPM8 (http://www.fil.ion.ucl.ac.uk/spm). The first 10 volumes for each participant were discarded to allow the signal to reach equilibrium and the participants to adapt to the scanning noise. The remaining volumes were corrected for the acquisition time delay between slices. Then, realignment was performed to correct for motion between time points. All participants’ BOLD data were within the defined motion thresholds (i.e., translational or rotational motion parameters <2 mm or 2°). We also calculated frame-wise displacement (FD), which indexes the volume-to-volume changes in the head position. Several nuisance covariates (six motion parameters, their 1st time derivative)

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**Table 1: Demographic and clinical characteristics of the sample**

| Characteristics      | AVH (n = 35) | nAVH (n = 41) | Controls (n = 50) | P |
|----------------------|--------------|---------------|-------------------|---|
| Age (years)          | 31.5 ± 7.7   | 32.3 ± 5.7    | 32.0 ± 8.2        | <0.005* |
| Sex (female/male)    | 17/24        | 14/21         | 18/32             | 0.857  |
| Antipsychotic dosage (mg/d) (chlorpromazine equivalents) | 518.1 ± 395.6 | 429.9 ± 259.5 | NA                | 0.248† |
| Duration of illness (months) | 101.0 ± 94.3 | 118.8 ± 71.7  | NA                | 0.363† |
| PANSS Total score    | 73.2 ± 23.6  | 68.4 ± 23.0   | NA                | 0.375† |
| Positive score       | 20.1 ± 7.7   | 14.8 ± 7.6    | NA                | 0.004† |
| Negative score       | 18.8 ± 8.2   | 20.2 ± 9.2    | NA                | 0.555† |
| General score        | 34.3 ± 11.5  | 33.6 ± 10.5   | NA                | 0.784† |
| AHRS total score     | 23.9 ± 8.4   |               |                   |       |

*One-way ANOVA was used to test the difference in age across the three groups; †Chi-square test was used to test the difference in gender across the three groups; ‡Two-sample t-test was used to compare the differences in antipsychotic dosage, duration of illness and PANSS scores between two patient groups. The data are shown as the mean values ± SD. NA: Not applicable; PANSS: Positive and negative syndrome scale; AHRS: Auditory hallucination rating scale; AVH: Schizophrenia patients with auditory verbal hallucinations; nAVH: Schizophrenia patients without auditory verbal hallucinations; SD: Standard deviation.
Regional homogeneity calculation

ReHo was defined as the Kendall correlation coefficient (KCC) of the time series of a given voxel with those of its nearest neighbors (26 voxels) on a voxel-wise basis. The KCC can be computed by the following formula:

\[ W = \frac{\sum (R_i^2 - \bar{R}^2)}{(1/12)K^2(n^2 - n)} \]

where \( W \) is the KCC among given voxels, ranging from 0 to 1; \( R_i \) is the sum rank of the \( i \)th time point; \( \bar{R} = [(n + 1)K] / 2 \) is the mean of \( R \); \( K \) is the number of time series within a measured cluster (\( K = 27 \), one given voxel plus its 26 neighbors), and \( n \) is the number of ranks (\( n = 240 \)). Then, each ReHo map was spatially smoothed with a Gaussian kernel of 6 mm × 6 mm × 6 mm full width at half maximum. Finally, we normalized the ReHo of each voxel by dividing it by the mean ReHo value of the whole brain.

Statistical analysis

Group differences in ReHo among the three groups were tested using a voxel-wise one-way analysis of covariance (ANCOVA) with age and gender as covariates followed by post hoc intergroup comparisons. The post hoc intergroup comparisons were conducted within a mask showing ReHo differences from the ANCOVA analysis.

In general, gender could not be considered as a covariance in the ANCOVA analysis; however, in our current study, our ANCOVA analysis was performed using the general linear model (GLM) implemented in SPM8. For GLM, categorical variable including gender should also be taken as independent variable. Hence, in this study, we used the gender as a covariance in our current ANCOVA analysis. Multiple comparisons were corrected using a false discovery rate (FDR) method with a significance threshold of \( P < 0.05 \).

Results

Demographic and clinical characteristics

Demographic and clinical data for the subjects are presented in Table 1. The three groups were well-matched in gender (Chi-square test, \( \chi^2 = 0.308, P = 0.857 \)) and age (one-way ANOVA, \( F = 0.100, P = 0.905 \)). There were no significant differences in antipsychotic dosage (two-sample \( t \)-test, \( t = 1.163, P = 0.248 \)), duration of illness (two sample \( t \)-test, \( t = -0.916, P = 0.363 \)), PANSS negative score (two sample \( t \)-test, \( t = -0.594, P = 0.555 \)), PANSS general score (two sample \( t \)-test, \( t = 0.275, P = 0.784 \)), or PANSS total score (two sample \( t \)-test, \( t = 0.893, P = 0.375 \)) between the AVH and the nAVH patients.

Regional homogeneity differences across groups

A voxel-wise ANCOVA revealed that the intergroup differences in ReHo were mainly located in the bilateral postcentral gyrus and thalamus, the left precuneus and putamen, and the right caudate, inferior temporal gyrus and inferior occipital gyrus (FDR corrected, \( P < 0.05 \)) [Figure 1]. Specifically, the AVH group exhibited significantly increased ReHo in the left precuneus relative to the nAVH group. Compared with the healthy controls, the AVH patients showed significantly increased ReHo in the left precuneus and putamen and the right caudate and inferior temporal gyrus and decreased ReHo in the bilateral postcentral gyrus and thalamus and the right inferior occipital gyrus (FDR corrected, \( P < 0.05 \)) [Figure 1]. In addition, the nAVH group had significantly increased ReHo in the right caudate and inferior temporal gyrus and decreased ReHo in the bilateral postcentral gyrus and thalamus and the right inferior occipital gyrus compared with the healthy controls (FDR corrected, \( P < 0.05 \)) [Figure 1].

Figure 1: Brain regions with altered ReHo across the AVH, nAVH, and control groups. One-way analysis of covariance and post hoc two-sample \( t \)-tests were used for intergroup comparisons (\( P < 0.05 \), false discovery rate corrected). AVH: Schizophrenia patients with auditory verbal hallucinations; nAVH: Schizophrenia patients without auditory verbal hallucinations; ReHo: Regional homogeneity.
Unfortunately, in brain regions demonstrating AVH-specific ReHo alterations, we did not find any statistical correlation between ReHo and ARHS score in the schizophrenia patients with AVHs.

**Discussion**

To the best of our knowledge, the current study is the first study to investigate the alteration of local brain spontaneous neural activity specific to AVHs in patients with schizophrenia by RS-fMRI using ReHo as an index. We found that ReHo in the right caudate and inferior temporal gyrus was higher in both schizophrenic patient groups than in the healthy controls whereas ReHo in the bilateral postcentral gyrus and thalamus and the right inferior occipital gyrus was lower in both schizophrenic patient groups (FDR corrected, $P < 0.05$). However, the key and novel finding of our current study is that the AVH group demonstrated significantly increased ReHo in the left precuneus compared with the nAVH group.

We found aberrant ReHo distributed in several brain regions in both schizophrenic patient groups, providing further evidence that local synchronization disturbances in the somatosensory cortex, visual processing related cortices, and some components of the limbic system may be the pathological features of schizophrenia. These synchronization disturbances were irrespective of the presence of AVH and were mostly consistent with our previous meta-analysis and large-sample study findings.[15]

Our finding that schizophrenia patients with AVH demonstrated higher ReHo in the left precuneus than the nAVH patients and the healthy controls, suggests that increased ReHo in the left precuneus may be a pathological feature exclusive to AVH in schizophrenia. The precuneus is the key component of the DMN,[15,24,25] and there is growing evidence that the DMN plays a pivotal role in cognitive, memory retrieval,[26] and self-referential processing[27] and also participates in the processing of monitor inner speech.[28,29] All of the aforementioned processes modulated by DMN are involved in the generation and monitoring of speech and have been associated with the experience of AVH.[15,24] More importantly, converging evidence suggests that functional alterations in the DMN play a key role in the generation of AVH.[15,30]

Recently, a new hypothesis that intrinsic DMN instability can account for the emergence of hallucination was proposed by Jardri et al.[15] According to this hypothesis, the DMN’s spatial and temporal instability is related to the severity of the hallucination; however, spatial instability exists only in the course of hallucination activity and is negatively correlated with the severity of the hallucination. Conversely, temporal instability exists in both the symptom-active and symptom-free course. More importantly, hyperpower spectral density (an index of the default mode component’s time course) in DMN is positively correlated with the severity of the hallucination.[15] The functional disability of DMN subsequent to the intrinsic instability influences the transition between resting and active conscious sensory states, hence the emergence of hallucination.[15] Our finding of increased ReHo in the left precuneus, one component of DMN, supports the hypothesis that intrinsic instability of the DMN participates in the generation of AVHs.

We did not find a correlation between the ReHo value of the left precuneus and the severity of auditory hallucinations in the AVH patients. This finding likely indicates that aberrant ReHo is a pathological feature of AVH schizophrenia, irrespective of its severity. This finding is partly consistent with the previous finding that the intrinsically temporal instability of DMN exists in both hallucination-active and -alleviated states.[15]

Some potential limitations must be noted when interpreting the results of the current study. First, the majority of the participating patients received antipsychotic drug treatment, and its effect on the ReHo in schizophrenia remain unclear. However, there was no significant difference in antipsychotic dosage between the schizophrenia patients with and without AVH, which can control for this confounder to some extent. Future studies focusing on first-episode drug-naive schizophrenia patients are needed to thoroughly control for this confounder. Second, we did not assess the status of hallucination symptoms during the MRI, which means that some patients likely experienced AVH, whereas others may not have, during the MRI course. Instead, we assessed AVH and other psychosis symptoms in all of the patients before the MRI procedure to improve our ability to precisely characterize the exclusive features of AVHs. This limitation can also be overcome in a future patient-specific study. Third, we enrolled patients who had not experienced AVH within 12 months before MRI scanning in the present study. These patients experienced auditory hallucinations only earlier in their psychotic illness or were in complete remission after treatment, which means that the findings of the present study probably do not comprehensively characterize the features of active hallucination when scanning. Fourth, we did not find a correlation between the ReHo value and the severity of AVH, likely due to the complexity of the relationship between local spontaneous neural activity and AVH severity beyond a simple linear correlation. Thus, more complex models, such as a quadratic regression model, can help us to clarify the relationship between them in a future study.

There are several limitations in this study. First, we did not collect the PANSS score of healthy subjects; hence, unfortunately, we could not not take the positive score as a covariate in the process of ANCOVA analysis. Second, similar to many previous studies, healthy controls did not accept antipsychotic administration; hence, we also did not take the psychotics dosage as a covariate in the ANCOVA analysis. In the future study, we will consider these factors in the process of statistics and provide more accurate information for enhancing the understanding of brain function alteration of schizophrenia.

Collectively, in this study, we found that schizophrenia patients with and without AVHs share common local spontaneous neural activity alterations in distributed
brain regions that participate in somatosensory and visual processing and some regions of the limbic system. More importantly, we found that only schizophrenia patients with AVH demonstrate increased ReHo in the pivotal component of the DMN (left precuneus). This finding provides new data for the development of hypotheses regarding schizophrenia AVHs and suggests that the intrinsic instability in the DMN is associated with the generation of AVHs.

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**Conflicts of interest**

There are no conflicts of interest.

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