

Original Research

# Altered Amplitude of Low-Frequency Fluctuations and Degree Centrality in Patients with Acute Subjective Tinnitus: A Resting-State Functional Magnetic Resonance Imaging Study

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## Abstract

**Background:** The difference in spontaneous brain activity between acute subjective tinnitus patients (with or without hearing loss) and control participants was explored using the amplitude of low-frequency fluctuations and degree centrality methods through resting-state functional magnetic resonance imaging. The study aimed to provide an objective basis for clinical diagnosis and pathogenesis of patients with acute subjective tinnitus. **Methods:** Fourteen acute subjective tinnitus (AST) patients with hearing loss (AST-HL), 6 AST patients with no hearing loss (AST-NHL), and 14 healthy controls (HCs) with age, sex, and education status matched were recruited for this study. Resting-state functional magnetic resonance imaging (fMRI) examinations were performed in a resting state and the amplitude of low-frequency fluctuations (ALFF) and degree centrality (DC) values of each group were acquired. Statistical analysis was performed to assess the ALFF and DC values of different brain areas of the participants (AST-HL and AST-NHL were compared with HCs, but AST-HL and AST-NHL were not). **Results:** Patients with acute subjective tinnitus and hearing loss showed a significantly increased amplitude of low-frequency fluctuation values in the left middle temporal gyrus and bilateral frontal gyrus/marginal lobe/cingulate gyrus but a decreased amplitude of low-frequency fluctuations values in the bilateral superior temporal gyrus/anterior cerebellar lobe in comparison with healthy controls. The amplitude of low-frequency fluctuation values of patients with acute subjective tinnitus and hearing loss in the right posterior lobe of the cerebellum, bilateral temporal gyrus, bilateral lenticular nucleus, bilateral frontal gyrus, right inferior occipital gyrus, were higher, but were significantly lower in the bilateral anterior lobe of cerebellum/superior temporal gyrus and left posterior cerebellar lobe as compared with those of healthy controls. Degree centrality values in the healthy controls group were increased in the right superior marginal gyrus and decreased in the right thalamus in patients with acute subjective tinnitus and hearing loss, while patients with acute subjective tinnitus with no hearing loss presented significantly higher degree centrality values in the left frontal lobe and lower degree centrality values in the left center rear return. **Conclusions:** Aberrant amplitude of low-frequency fluctuations and values exist in various brain regions, indicating abnormal spontaneous brain activity in both acute subjective tinnitus and hearing loss and acute subjective tinnitus no hearing loss patients. The pathogenesis of acute subjective tinnitus may be related to abnormalities in both the auditory cortex and nonauditory cortex. These findings provide more evidence to help clarify the neuronal symptoms of acute subjective tinnitus patients.

**Keywords:** acute subjective tinnitus; hearing loss; amplitude of low-frequency fluctuations; degree centrality

## 1. Introduction

Tinnitus, a common hearing disease, can be subdivided into subjective tinnitus and objective tinnitus. Subjective tinnitus is often observed in clinical settings, while objective tinnitus is rare. Without any corresponding external auditory source, tinnitus can be expressed by affected patients in a variety of ways including ringing, buzzing, hissing, or clicking. Additionally, tinnitus can be accompanied by different degrees of hearing loss. The prevalence of tinnitus is about 10% to 15% in humans [1] and the incidence rate of tinnitus increases with age, so it is more common among adults older than 65 years [2]. Tinnitus

has become the third most harmful otological disease after deafness and vertigo. According to the 2014 United States tinnitus Clinical Application Guidelines, acute subjective tinnitus (AST) refers to tinnitus with an onset time of no more than six months. Long-term tinnitus can have a serious impact on patients, who may also suffer from sleep disorder, inattention and emotional disorder. As compared with chronic tinnitus, patients with AST are more motivated to seek clinical help. Currently, the pathogenesis of tinnitus remains unclear, and effective objective examinations and tailored medicines for tinnitus are rare, causing difficulties for clinical treatment and nursing.



The mechanism of tinnitus has been studied for many years but has yet not been fully elucidated. Previously, it was generally believed that tinnitus occurs when the peripheral auditory system was acutely or chronically damaged or underwent pathological changes. Recently it has been found that tinnitus may continue to exist even if the auditory nerve is severed to block the input of sound to the ear, so tinnitus is not only linked to a damaged cochlea [1]. More recently, with the progression of basic research using tinnitus animal models, an updated theory of tinnitus centralization has been put forward: abnormal central neuronal activity and reorganization of the central cortex or subcortical structure may be the cause of tinnitus. Furthermore, nonauditory centers including the limbic system and autonomic nervous system may also participate in tinnitus.

The rapid development of neuroimaging and neuroelectrophysiology has made it possible to gather detailed evidence verifying recently updated theory. Resting-state functional magnetic resonance imaging (fMRI) has the advantages of being an objective and repeatable data acquisition technique. It displays the brain function activities of specific areas and is a powerful tool for observing auditory activities. The amplitude of low-frequency fluctuations (ALFF) is one type of fMRI data that measures the internal average response of the brain to indicate the intensity of local neuron activity. Specifically, it reflects the level of spontaneous activity of each single voxel from the perspective of energy and has been demonstrated to be an effective method for the evaluation of spontaneous changes in brain activity associated with various neurological or mental diseases [3]. The degree centrality (DC) value is the most direct index available to describe node centrality in a network analysis. The higher the DC value of a node, the more important that node is in the network. The DC method reflects the functional connection of the human brain at the voxel level. It has been successfully used to assess the pathological mechanisms of many diseases, such as primary open-angle glaucoma [4], schizophrenia with auditory hallucinations [5] and Parkinson's disease [6].

Previous studies have shown that tinnitus might be correlated with abnormal spontaneous neuronal activity in the auditory and nonauditory centers (frontal lobe, marginal lobe and cerebellum) [7–9]. However, previously published results have varied widely, due to differences in the fMRI techniques and calculation methods applied. In the current study, ALFF and DC were used to analyze the brain function activities of patients with tinnitus. These two techniques can directly measure the intensity and connection state of neural activity between brain regions, and have high test-retest reliability. Therefore, the combination of these two methods may be more realistic than using either method alone. The ALFF and DC methods were employed to detect spontaneous brain activity in patients with AST to clarify the neuropathological mechanism of tinnitus-related symptoms.

## 2. Materials and Methods

### 2.1 Subjects

The present study was approved by the Medical Ethics Committee of Nanchang University. Fourteen AST patients with hearing loss (AST-HL) and six AST patients with no hearing loss (AST-NHL) were included in the study alongside 14 healthy controls (HCs) who were matched to the AST patients according to age, sex and education state.

The inclusion criteria for patients with AST-HL and those with AST-NHL were as follows: (1) No neurologic or psychiatric history or standard MRI contraindications; (2) No organic tinnitus, myogenic tinnitus, vascular tinnitus, or certain systemic diseases such as diabetes, acoustic neuroma, Meniere's disease, or unilateral cochlear injury caused by head trauma, infection, or hemorrhage; (3) An onset time of tinnitus of no more than six months prior; (4) Right-handed; (5) The ability to undergo pure tone audiometry at frequencies of 125, 250, 500, 1 K, 2 K, 4 K and 8 kHz, with an average hearing threshold of 0.125 to 8 kHz being more than 30 dB or not more than 30 dB in all patients; (6) A tinnitus diagnosis confirmed by the main complaint, history inquiry, general clinical examination, audiology test and tinnitus examination according to the 2015 Tinnitus Clinical Application Guidance; and (7) An fMRI examination performed after admission before any treatment. All AST patients were asked to complete the Tinnitus Handicap Inventory (THI) to assess the severity of their tinnitus; the higher the total score (0–100 points), the greater the impact of tinnitus on daily life.

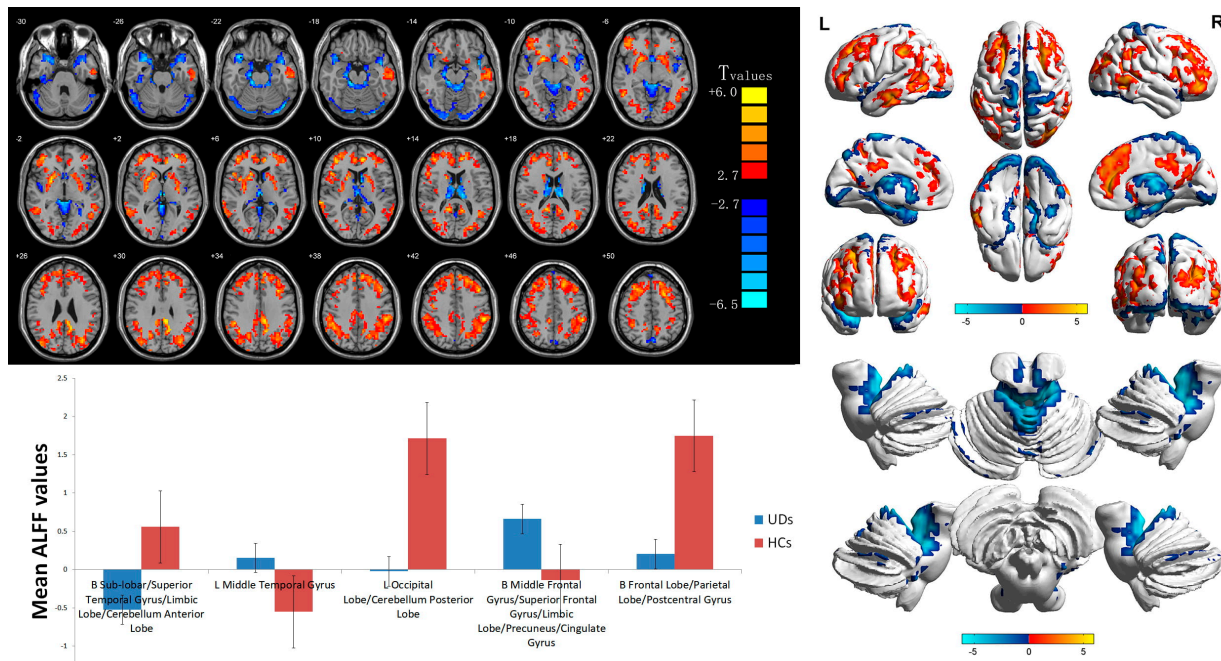
During the same period, 14 HCs were recruited who met the following criteria: (1) No tinnitus, vertigo, hearing loss, or other vestibular diseases; (2) Normal otology and audiology examination findings; (3) No system or psychiatric diseases; (4) Right-handed; and (5) No contraindications for MRI scanning.

### 2.2 MRI Data Acquisition

All participants underwent MRI scanning using a 3.0-Tesla Verio scanner (Siemens, Erlangen, Germany) at the First Affiliated Hospital of Nanchang University. During the scanning process, patients were asked to remain quiet with their eyes closed. During imaging, gradient-echo echo-planar imaging sequence was performed with the following parameters: repetition time 2000 ms, echo time 40 ms, flip angle 90°, slice thickness 4 mm, matrix size 64 × 64 and field of view 240 mm × 240 mm. A total of 240 volumes were obtained. Additionally, T1-weighted imaging was performed with the following parameters: repetition time 1900 ms, echo time 2.26 ms, flip angle 9°, slice thickness 1 mm, matrix size 256 × 256, field of view 240 mm × 240 mm and an acquisition time of three minutes.

### 2.3 fMRI Data Preprocessing

Bold data were processed using SPM8 software (<http://www.fil.ion.ucl.ac.uk/spm>; Wellcome Centre for



**Fig. 1. Voxelwise comparison of ALFF values between AST-HL and HC groups (two-dimensional sections).** Brain clusters show a significant difference in ALFF values between AST-HL and HC groups (cluster-level  $p < 0.001$ , GRF correction). Hot/cool colors indicate significantly increased/decreased ALFF values in the AST-HL group.

Human Neuroimaging, UCL Queen Square Institute of Neurology, London, UK) on the MATLAB platform (MathWorks Inc, Natick, MA, USA). Specific procedures were as follows: (1) The first 10 volumes were removed for each subject and the remaining 230 useful volumes were corrected for slice timing and motion correction, (2) The data underwent spatial standardization according to the Montreal Neurological Institute (MNI) template (resampling voxel size of  $3 \times 3 \times 3 \text{ mm}^3$ ), (3) Patient data with head translation of greater than 2 mm and/or rotation of greater than  $2^\circ$  in any direction and/or signals of whole-brain white matter and cerebrospinal fluid were excluded, (4) Covariates (six head motion parameters, mean framewise displacement (FD), global brain signal and the average signal from white matter signal and cerebrospinal fluid) were used to regress out.

Data Processing & Analysis for Brain Imaging software was used to calculate ALFF and DC values and resting-state data analysis was adopted for the purposes of statistical analysis.

#### 2.4 ALFF Analysis

The ALFF value was obtained with the following steps. First, a 6 mm full-width-at-half-maximum Gaussian kernel was used to smooth the z-score map, each voxel ALFF value of each frequency point was divided by the average value of the whole-brain ALFF value to obtain a standardized ALFF value. Spatial smoothing was then used to correct and reduce the errors with the Gaussian random field (GRF). Finally, a comparison between groups was com-

pleted with the SPM8 software. Clusters with more than 26 adjacent suprathreshold voxels were selected as the regions with statistically significant differences between the two groups ( $p < 0.001$ ; cluster  $> 26$ ).

#### 2.5 DC Analysis

The voxel-wise functional network was generated for each subject, for which we took each voxel as a node and inter-voxel correlations as the edge. Within the default brain mask provided by DPARSFA (in the MNI-152 standard space with  $3 \times 3 \times 3 \text{ mm}^3$  voxel size and resolution of  $61 \times 73 \times 61$ ), we used the preprocessed functional images to perform a voxel-wise correlation analysis. For each subject, we calculated Pearson's correlation between the time courses for any pair of voxels, resulting in a 70831-by-70831 correlation matrix. An undirected adjacency matrix was then obtained by setting a threshold to each correlation at  $r > 0.25$  [10]. Based on the individual voxel-wise functional network, DC was calculated by counting the number of significant suprathresholded correlations (or the degree of the binarized adjacency matrix) for each subject. The voxel-wise DC map for each individual was converted into a z-score map using the following equation:

$$Z_i = \frac{DC_i - \text{mean}(DC \text{ of all voxels in brain mask})}{\text{std}(DC \text{ of all voxels in brain mask})}$$

where  $i$  is the voxel index,  $DC_i$  is the DC value for the  $i$ -th voxel,  $\text{std}$  is the standard deviation, and  $Z_i$  is the z-score for the  $i$ -th voxel. The z-score map was spatially smoothed with a Gaussian kernel of FWHM (full width at half maximum) of 6 mm.

**Table 1. Characteristics of study participants.**

	AST-HL	AST-NHL	HC	<i>p</i> -value	<i>p</i> -value
Sex (male/female)	7/7	3/3	7/7	–	–
Age (years)	33.3 ± 12.3	26.85 ± 9.92	27.6 ± 10.6	0.214	0.285
Handedness	14R	6R	14R	–	–
Education state (years)	13.9 ± 4.15	12.6 ± 3.35	14.5 ± 3.26	0.384	0.391
Tinnitus laterality (right/left)	6/8	0/6	–	–	–
PTA of right ear (dB)	53.5 ± 13.4	–	15.2 ± 1.54	<0.001	/
PTA of left ear (dB)	60 ± 15.8	15.2 ± 1.81	15.6 ± 1.62	<0.001	0.601
Duration (days)	3.35 ± 2.44	37.5 ± 28.59	–	–	0.007
THI score	69.7 ± 11.58	42 ± 25.83	–	–	0.003

AST, acute subjective tinnitus; HC, healthy control; AST-HL, AST patients with hearing loss; AST-NHL, AST patients with no hearing loss; R, right; THI, Tinnitus Handicap Inventory. A chi-squared test for sex and handedness data and an independent *t*-test for continuous data were used.

**Table 2. Comparison of ALFF values between AST-HL and HC groups.**

Brain area	Peak MNI coordinates			Cluster voxel	BA	L/R/B	<i>t</i> -value
	<i>x</i>	<i>y</i>	<i>z</i>				
AST-HL > HCs							
Middle temporal gyrus	−60	−18	−18	232	21	L	4.7469
Middle frontal gyrus/superior frontal gyrus/marginal lobe/anterior uncinate gyrus/cingulate gyrus	21	12	−9	7120	40	B	5.9543
AST-HL < HCs							
Superior temporal gyrus/anterior cerebellar lobe	15	−30	−18	2442	38	B	−6.0499
Posterior cerebellar lobe	−45	−75	−21	216	18	L	−6.1371
Posterior central gyrus	3	−24	78	549	6	B	−5.6494

MNI, Montreal Neurological Institute; BA, Brodmann area. (*x*, *y*, *z*) Coordinates of the peak location in the MNI space, *t* statistical value of the peak voxel.

## 2.6 Statistical Analysis

The Statistical Package for the Social Sciences version 22.0 software (IBM Corporation, Armonk, NY, USA) was used to compare differences between groups (age, years of education, listening). The chi-squared test was used to compare the classification data (handedness and sex). A two-sample *t*-test was conducted to test the difference in *z*-scores between AST-HL or AST-NHL and HC groups. A *p*-value of less than 0.05 was taken to indicate significant difference.

## 3. Results

### 3.1 Demographic and Behavior Measurements

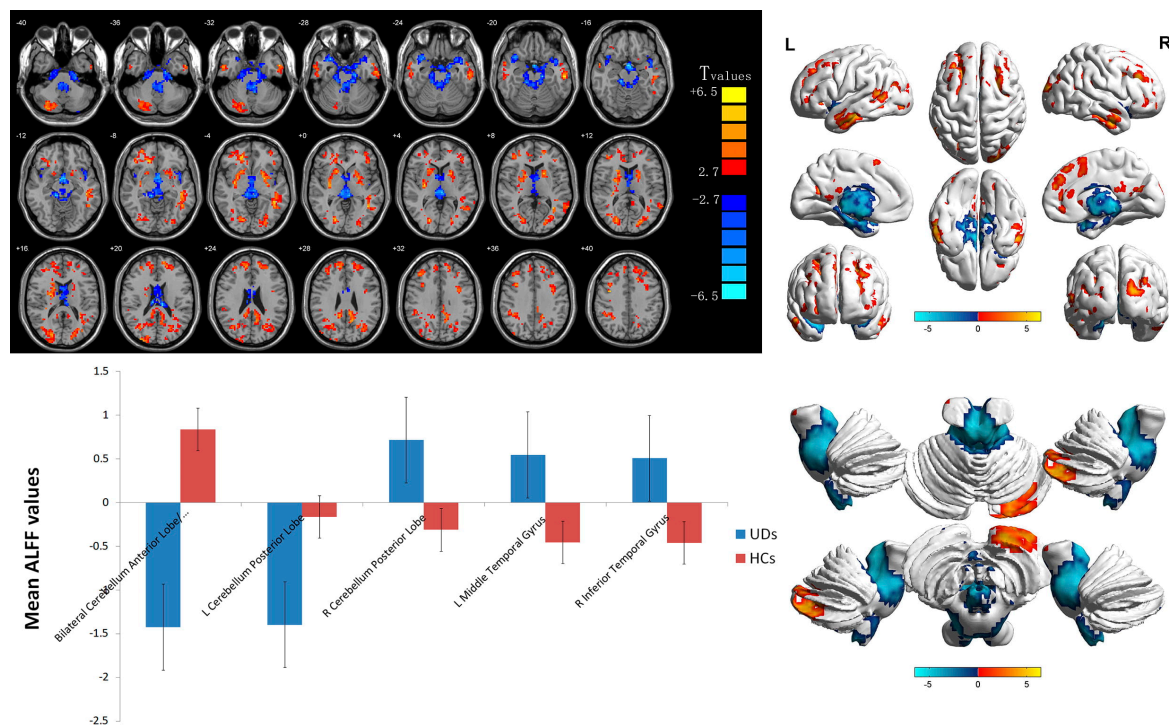
No significant difference was found in terms of sex, age, education state or handedness between AST-HL or AST-NHL patients and HCs (see Table 1). In the AST-HL group, six cases occurred in the right ear where the average hearing threshold was 53.5 ± 13.4 dB, while eight cases occurred in the left ear where the average hearing threshold was 60 ± 15.8 dB. The contralateral ears of all patients were normal (see Table 1). However, all AST-NHL patients had tinnitus in the left ear. Comparisons between the two groups showed no significant group differences in the average hearing threshold for both the worst (*p* = 0.601, see Table 1). The THI scores of AST-HL and AST-NHL were

69.7 ± 11.58 points and 42 ± 25.83 points (*p* = 0.003), the duration (days) of AST-HL and AST-NHL were 3.35 ± 2.44 and 37.5 ± 28.59 (*p* = 0.007) respectively (see Table 1).

### 3.2 Analysis of ALFF and DC

As compared with the HC group, the AST-HL group showed significantly increased ALFF values in the left middle temporal gyrus cluster voxel = 232 (*t* = 4.7469) and bilateral middle frontal gyrus/superior frontal gyrus/marginal lobe/anterior uncinate gyrus/cingulate gyrus cluster voxel = 712 (*t* = 5.9543) but decreased ALFF values in the bilateral superior temporal gyrus/anterior cerebellar lobe cluster voxel = 2442 (*t* = –6.050) left posterior cerebellar lobe cluster voxel = 216 (*t* = –6.137) bilateral posterior central gyrus cluster voxel = 549 (*t* = –5.649) see Table 2 and Fig. 1. As seen in Table 3 and Fig. 2 post hoc comparison of ALFF scores between AST-NHL and the combined HCs group, using a two-sample *t*-test, resulted in eleven clusters of increased ALFF scores in the AST-NHL group (the right posterior lobe of the cerebellum cluster voxel = 210 (*t* = 5.275), left middle temporal gyrus cluster voxel = 1140 (*t* = 6.451), right inferior temporal gyrus cluster voxel = 106 (*t* = 5.218), left lenticular nucleus cluster voxel = 194 (*t* = 5.633), right middle frontal gyrus cluster voxel = 375 (*t* = 6.410), right





**Fig. 2. Voxelwise comparison of ALFF values between AST-NHL and HC groups (two-dimensional sections).** Brain clusters show a significant difference in ALFF values between the two groups (cluster-level  $p < 0.001$ , GRF correction). Hot/cool colors indicate significantly increased/decreased ALFF values in the AST-NHL group.

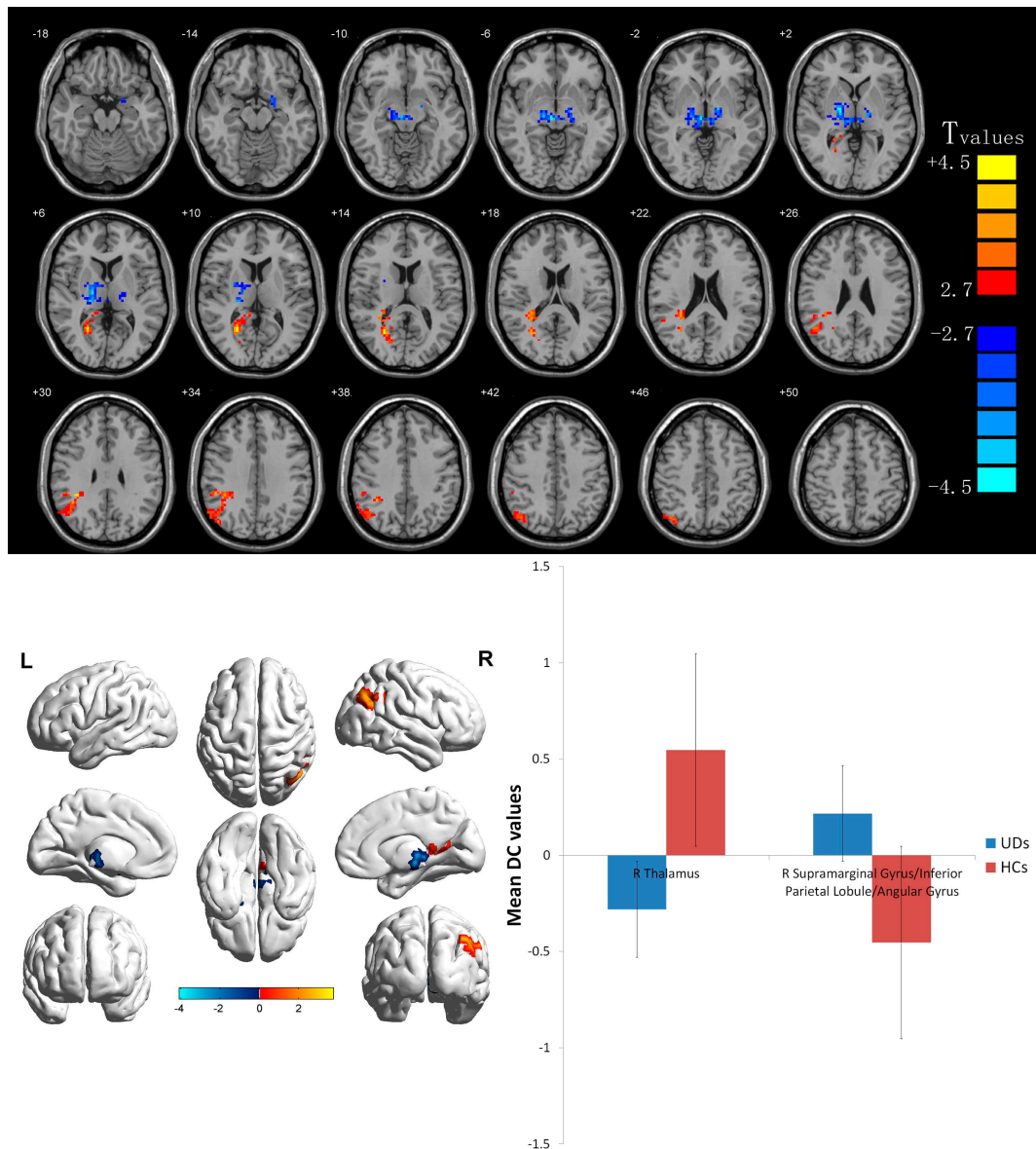
**Table 3. Comparison of ALFF values between AST-NHL and HC groups.**

Brain area	Peak MNI coordinates			Cluster voxel	BA	L/R/B	<i>t</i> -value
	<i>x</i>	<i>y</i>	<i>z</i>				
AST-NHL > HC							
Posterior lobe of cerebellum	30	−84	−39	210	−	R	5.2752
Middle temporal gyrus	−45	−48	−3	1140	7	L	6.4505
Inferior temporal gyrus	54	−6	−30	106	21	R	5.2177
Lenticular nucleus	−24	0	−3	194	47	L	5.6326
Middle frontal gyrus	15	33	−6	375	10	R	6.4096
Lenticular nucleus	30	−12	3	312	−	R	5.6533
Inferior occipital gyrus	21	−81	0	549	19	R	5.4516
Inferior frontal gyrus	−42	36	3	72	46	L	4.8373
Superior frontal gyrus	−18	24	45	538	8	L	6.2018
Medial frontal gyrus	9	48	30	184	9	R	5.3571
Middle frontal gyrus	9	18	51	328	6	R	5.9842
AST-NHL < HCs							
Anterior lobe of cerebellum/midbrain/superior temporal gyrus	0	0	−18	2731	−	B	−6.493
Posterior cerebellar lobe	−3	−75	−48	188	−	L	−5.9085

MNI, Montreal Neurological Institute; BA, Brodmann area. (*x*, *y*, *z*), coordinates of the peak location in the MNI space, *t* statistical value of the peak voxel.

lenticular nucleus cluster voxel = 312 ( $t = 5.653$ ), right inferior occipital gyrus cluster voxel = 549 ( $t = 5.452$ ), left inferior frontal gyrus cluster voxel = 72 ( $t = 4.837$ ), left superior frontal gyrus cluster voxel = 538 ( $t = 6.202$ ), right medial frontal gyrus cluster voxel = 184 ( $t = 5.357$ ), and right middle frontal gyrus cluster voxel = 328 ( $t = 5.984$ ). Conversely, ALFF values in the bilateral anterior lobe of the

cerebellum/midbrain/superior temporal gyrus cluster voxel = 2731 ( $t = -6.493$ ) and left posterior cerebellar lobe cluster voxel = 188 ( $t = -5.909$ ) in AST-NHL patients were significantly lower than those of HCs. However, as compared with among HCs, DC values were increased in the right superior marginal gyrus/inferior parietal lobule/angular gyrus cluster voxel = 384 ( $t = 3.784$ ) and decreased in the right



**Fig. 3. Voxelwise comparison of DC values between AST-HL and HC groups (two-dimensional sections).** Brain clusters show a significant difference in DC values between these two groups (cluster-level  $p < 0.05$ , Alphasim correction). Hot/cool colors indicate significantly increased/decreased DC values in the AST-HL group.

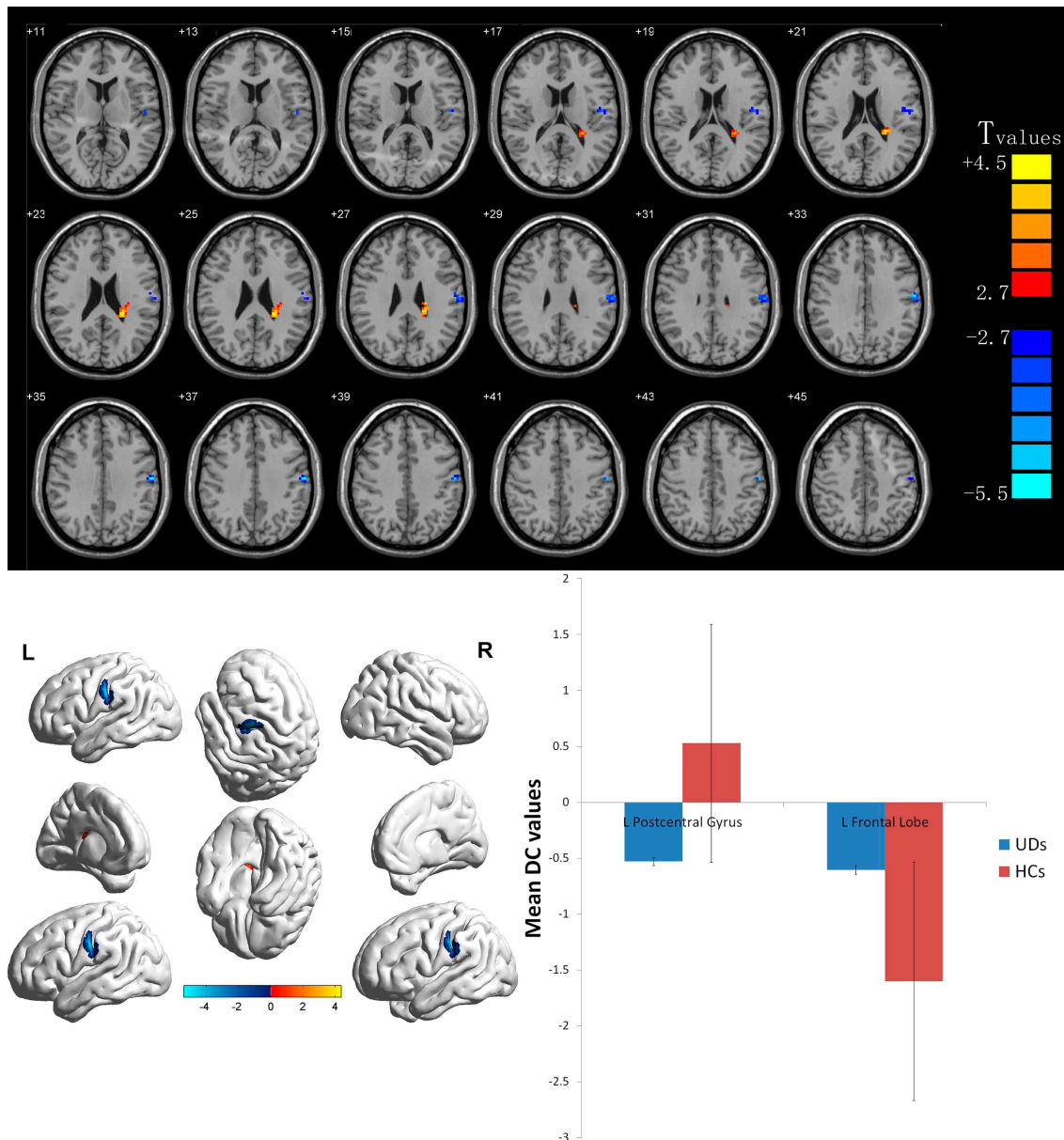
**Table 4. Comparison of DC values between AST-HL and HC groups.**

Brain area	Peak MNI coordinates			Cluster voxel	BA	L/R/B	<i>t</i> -value
	<i>x</i>	<i>y</i>	<i>z</i>				
AST-HL > HCs							
Superior marginal gyrus/inferior parietal lobule/angular gyrus	27	−60	12	384	39	R	3.7843
AST-HL < HCs							
Thalamus	18	−9	3	316	34	R	−4.1048

MNI, Montreal Neurological Institute; BA, Brodmann area. ( $x, y, z$ ) coordinates of the peak location in the MNI space,  $t$  statistical value of the peak voxel.

thalamus cluster voxel = 316 ( $t = -4.105$ ) in AST-HL patients (shown in Table 4 and Fig. 3). Additionally, AST-NHL patients displayed significantly higher DC values in the left frontal lobe cluster voxel = 43 ( $t = 4.380$ ) and lower

DC values in the left center rear return cluster voxel = 64 ( $t = -5.403$ ) (see Table 5 and Fig. 4).



**Fig. 4. Voxelwise comparison of DC values between AST-NHL and HC groups (two-dimensional sections).** Brain clusters show a significant difference in DC values between the two groups (cluster-level  $p < 0.01$ , Alphasim correction). Hot/cool colors indicate significantly increased/decreased DC values in the AST-NHL group.

**Table 5. Comparison of DC values between AST-NHL and HC groups.**

Brain area	Peak MNI coordinates			Cluster voxel	BA	L/R/B	<i>t</i> -value
	<i>x</i>	<i>y</i>	<i>z</i>				
AST-NHL > HCs							
Frontal lobe	−24	−36	21	43	–	L	4.3801
AST-NHL < HCs							
Center rear return	−60	−15	33	64	3	L	−5.4032

MNI, Montreal Neurological Institute; BA, Brodmann area. ( $x, y, z$ ) coordinates of the peak location in the MNI space,  $t$  statistical value of the peak voxel.

## 4. Discussion

The current study revealed findings of altered brain activity related to AST patients for the first time by combining ALFF and DC analyses. Specifically, extensive brain regions were identified that belonged to the auditory network, default mode network and visual network that presented remarkably different ALFF and DC values in the study population. More generally, the reported findings support that neuroimaging may be useful for detecting the characteristics of neural network modifications in patients with AST by indicating the neuropathological mechanism of tinnitus.

The auditory cortex is divided into the primary and secondary cortices. This study reported increased ALFF



values in the middle temporal gyrus (secondary auditory cortex) and decreased ALFF values in the superior temporal gyrus (primary auditory cortex) in both the AST-HL and AST-NHL groups. In a positron-emission tomography study, a correlation between higher brain metabolism in the middle temporal gyrus and tinnitus severity was confirmed [11]. Further, Chen *et al.* [9] recorded increased ALFF values in the right middle temporal gyrus of patients with chronic tinnitus. Additionally, a greater level of tinnitus disturbance was correlated with increased ALFF values in the middle temporal gyrus, suggesting that a change in the ALFF value may reflect an influence of tinnitus perceptions. Previous studies have demonstrated that the volume of the bilateral superior temporal gyrus in patients with unilateral pulsatile tinnitus was significantly larger than that in the normal control group, while FC in both the right superior temporal gyrus and left inferior temporal gyrus was abnormally increased [12,13]. Contrary to the above results, the ALFF value of the superior temporal gyrus was significantly reduced in this study, suggesting dysfunction of the superior temporal gyrus. Therefore, it is speculated that the mechanism of tinnitus might be linked to activation of the secondary auditory center and inhibition of the primary auditory center.

The superior frontal gyrus and middle frontal gyrus may be key nodes in the tinnitus network responsible for receiving and integrating tinnitus sensory information and transmitting the impulses to maintain the overall coordination of the central nervous system. Geven [14] found an enhancement metabolism of the left middle frontal gyrus in tinnitus patients using 2-deoxy-2- (fluorine-18) fluorodeoxyglucose positron-emission tomography. Additionally, a correlation between high neural activity in the middle frontal gyrus and the severity of tinnitus was observed [15]. The results of task-state fMRI also revealed an abnormal degree of activation of the superior frontal gyrus in tinnitus patients [16]. In chronic tinnitus patients, the FC of the left middle frontal gyrus was also significantly increased [17]. Consistent with previous studies, the findings reported here included significantly higher ALFF values from the superior and middle frontal gyrus in both AST-HL and AST-NHL patients. However, a previous study has shown that regional homogeneity (ReHo) values in several brain regions of the tinnitus group, including the inferior frontal gyrus, were increased [18]. Increased ALFF values were also observed in this study in the inferior frontal gyrus in the AST-NHL group. Acting as the core brain area of the central inhibitory response, the inferior frontal gyrus might reflect the presence of long-term control of central attention allocation to tinnitus perception from the bottom to top. It has been hypothesized that a default mode network (DMN) exists in the human brain, with the prefrontal gyrus holding an important role [19]. The DMN is involved in scene memory, self-evaluation and internal expression. Schmidt *et al.* [20] have also reported specific changes in patients

with tinnitus, including connectivity alterations in the DMN and dorsal attention network. They speculated that DMN might play a key role in the neurophysiological process of tinnitus. Taken together, tinnitus patients show extensive abnormally spontaneous brain activity in the frontal lobe, indicating that the frontal cortex may be a participant in the pathogenesis of tinnitus.

The limbic system and frontal lobe are the main brain areas that participate in emotion processing. However, studies are increasingly pointing to the fact that tinnitus is not only limited to the auditory system but also closely related to the psychological activities of emotion and memory. Boyen [21] contended that gray matter in the marginal lobe of tinnitus patients with hearing loss was increased. Significantly greater ReHo values were also found in tinnitus patients, related to the increased tinnitus duration and hearing threshold of tinnitus [22]. In this study, abnormal ALFF values were obtained in the marginal lobe, anterior uncinatus gyrus and cingulate gyrus, which might be related to psychological problems linked to stress, anxiety, inattention and insomnia present in AST patients. From an anatomical perspective, there was an indirect fiber connection observed between the auditory cortex and the limbic system, which allowed for a new model for the pathophysiology of tinnitus. In this context, the abnormal activity of the auditory system is transmitted to the brain and experienced as tinnitus. Simultaneously, negative experiences such as fidgeting, insomnia, depression and fear are generated due to the involvement of the limbic system and induce the perception of tinnitus, resulting in an abnormal cycle between tinnitus and negative characteristics or feelings.

The cerebellum has always been considered as the center of coordination for the random movement and contraction of muscles in the whole body. However, a recent study showed that the cerebellum also plays an important role in auditory afferent processing [23]. Bauer [24] observed that the cerebellum might be an important and non-mandatory site of tinnitus in a rat model of noise-induced chronic tinnitus. Once cerebellum lesions occur, tinnitus signals might appear. Tinnitus patients additionally showed significantly decreased ReHo values in the anterior lobe of the cerebellum [25]. Han [26] recorded altered ALFF values in the posterior lobe of the right cerebellum in pulsatile tinnitus patients in a manner linked to the diversity and duration of tinnitus. The results reported here indicate that ALFF values in the left posterior cerebellar lobe and bilateral anterior lobe of cerebellum were decreased in patients with tinnitus, which confirmed that the integration of cerebellar functional processing of cochlear afferent signals might be involved in the occurrence and persistence of tinnitus.

In the AST-NHL group, it was observed that the visual and extrapyramidal systems were activated. As an important part of the visual cortex, the inferior occipital gyrus is involved in logic analysis in the brain and the processing of



visual memory. Based on the anatomic connection between auditory and visual areas, Ibrahim [27] suggested that the auditory cortex directly regulates the visual cortex. Therefore, the current authors speculated that higher ALFF values in the right suboccipital gyrus in AST-NHL patients might be the result of the activation of the visual center triggered by tinnitus. The above result was consistent with that of Zhou *et al.* [2]. However, it was found instead that the central visual system was not activated in the AST-HL group, which might be due to the time of onset or degree of hearing loss in the current study population. This study also reported increased ALFF values in the bilateral lenticular nucleus of the AST-NHL group. The lenticular nucleus belongs to the striatum, which receives almost all projections from the cerebral cortex and has direct fiber connections with the auditory cortex and the medial geniculate body. As such, changes in the cortical striatal pathway might constitute an important factor in tinnitus. However, the specific role of the lenticular nucleus in tinnitus requires further investigation.

The DC values in the right superior marginal gyrus/inferior parietal lobule/angular gyrus were increased in AST-HL patients, suggesting that the DMN plays an important role in tinnitus. The DMN influences negative emotions and attention disorders among patients. Therefore, it was speculated that the clinical manifestations of anxiety and insomnia in these patients might be correlated with increased activity of the DMN and limbic system network, causing a damaging cycle between tinnitus and negative events or feelings, as described above. The thalamus has also been found to be essential for the processing of tinnitus [28]. As an important component of many pathways of the brain, the thalamus transmits sensory information to the cerebral cortex and is the most important hub of sensory transmission. Except for olfaction, all other sensory pathway transmission neurons in the body are managed in the thalamus. Llinás proposed a model of thalamic cortical rhythm disorder that suggested that tinnitus was caused by the interruption of coherent oscillation between the thalamus and cortex [29]. Impaired white matter integrity involving thalamic connectivity was previously identified in tinnitus patients [30]. Other studies have also observed decreased short-range FC density and ALFF values in the thalamus [9,31]. Consistent with the foregoing results, this study recorded significantly decreased DC values of the right thalamus in the AST-HL group, speculated to be caused by hearing loss—that is, in AST-HL patients, decreased auditory information stimulation caused weakened thalamo-cortical connections, thus leading to changes in the normal rhythm of the thalamo-cortical ring and ultimately, the generation of tinnitus.

As described above, the frontal gyrus was widely activated in tinnitus. Significantly higher DC values were obtained from the frontal gyrus of AST-NHL patients, which reinforced the fact that the frontal lobe plays an indispen-

able role in tinnitus. The posterior central gyrus, functioning as the primary somatosensory cortex, has been referred to as the joint area together with the frontal lobe and temporal lobe [32]. In this study, remarkably lower ALFF values were observed in the posterior central gyrus in AST-HL patients and decreased DC values were observed in the posterior central gyrus in AST-NHL patients. The dysfunction of the posterior central gyrus might be associated with sensory abnormalities in tinnitus patients.

### Limitations

First, this study was based on temporal sequences and the sample size was relatively small, so larger subject groups should be studied in the future. Second, patients with tinnitus on either the left or right side were included in this study, which does not explain the lateral problems of brain function. Third, the noise during fMRI scanning was quite loud. Although measures were taken, such as wearing earmuffs, this may not have totally eliminated this influence on the experimental results.

## 5. Conclusions

The results reported here confirmed that the auditory and nonauditory centers of tinnitus patients showed abnormal ALFF and DC values, indicating that both the auditory center and nonauditory centers (limbic system, frontal lobe, cerebellum, posterior central gyrus) are crucial for tinnitus. The neural activity of tinnitus brain area is related to the disease stage, suggesting that we should pay attention to the different stages of tinnitus disease; The abnormality of tinnitus brain network is mainly manifested in the enhancement of frontal lobe centrality and the decrease of posterior central gyrus centrality. fMRI data was employed to compare the abnormal activity brain area of tinnitus patients in a resting state, which is proposed to reflect the neural mechanism of tinnitus relatively objectively. The relationship between tinnitus and spontaneous brain activity of the auditory cortex, DMN, limbic system and attention network was revealed first by combining the ALFF and DC methods. In short, the current study provides details on a comprehensive neurological pattern in patients with AST.

### Author Contributions

JC, LF, carried out the experiments and wrote the manuscripts. GC wrote and edited manuscript and performed f-MRI experiments. YX, ZZ conceived the study, designed experiments and wrote the manuscript. BH, JC performed the acquisition, analysis and interpretation of data.

### Ethics Approval and Consent to Participate

Medical research ethics committee of the First Affiliated Hospital of Nanchang University (2019) Yi Yan Lun Shen No. (100).

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## Conflict of Interest

The authors declare no conflict of interest.

## References

- [1] Baguley D, McFerran D, Hall D. Tinnitus. *The Lancet*. 2013; 382: 1600–1607.
- [2] Zhou G, Shi X, Wei H, Qu L, Yu Y, Zhou Q, *et al*. Disrupted Intraregional Brain Activity and Functional Connectivity in Unilateral Acute Tinnitus Patients with Hearing Loss. *Frontiers in Neuroscience*. 2019; 13: 1010.
- [3] Hoptman MJ, Zuo X, Butler PD, Javitt DC, D'Angelo D, Mauro CJ, *et al*. Amplitude of low-frequency oscillations in schizophrenia: a resting state fMRI study. *Schizophrenia Research*. 2010; 117: 13–20.
- [4] Zhang Q, Shu Y, Li X, Xiong C, Li P, Pang Y, *et al*. Resting-state functional magnetic resonance study of primary open-angle glaucoma based on voxelwise brain network degree centrality. *Neuroscience Letters*. 2019; 712: 134500.
- [5] Chen C, Wang H, Wu S, Huang H, Zou J, Chen J, *et al*. Abnormal Degree Centrality of Bilateral Putamen and Left Superior Frontal Gyrus in Schizophrenia with Auditory Hallucinations: a resting-state functional magnetic resonance imaging study. *Chinese Medical Journal*. 2015; 128: 3178–3184.
- [6] Lou Y, Huang P, Li D, Cen Z, Wang B, Gao J, *et al*. Altered brain network centrality in depressed Parkinson's disease patients. *Movement Disorders*. 2016; 30: 1777–1784.
- [7] Kim J, Kim Y, Lee S, Seo J, Song H, Cho JH, *et al*. Alteration of functional connectivity in tinnitus brain revealed by resting-state fMRI? A pilot study. *International Journal of Audiology*. 2012; 51: 413–417.
- [8] Leaver AM, Turesky TK, Seydell-Greenwald A, Morgan S, Kim HJ, Rauschecker JP. Intrinsic network activity in tinnitus investigated using functional MRI. *Human Brain Mapping*. 2016; 37: 2717–2735.
- [9] Chen Y, Zhang J, Li X, Xia W, Feng X, Gao B, *et al*. Aberrant spontaneous brain activity in chronic tinnitus patients revealed by resting-state functional MRI. *NeuroImage: Clinical*. 2014; 6: 222–228.
- [10] Cai F, Gao L, Gong H, Jiang F, Pei C, Zhang X, *et al*. Network Centrality of Resting-State fMRI in Primary Angle-Closure Glaucoma Before and After Surgery. *PLoS ONE*. 2015; 10: e0141389.
- [11] Plewnia C, Reimold M, Najib A, Brehm B, Reischl G, Plontke SK, *et al*. Dose-dependent attenuation of auditory phantom perception (tinnitus) by PET-guided repetitive transcranial magnetic stimulation. *Human Brain Mapping*. 2010; 28: 238–246.
- [12] Liu Y, Lv H, Zhao P, Liu Z, Chen W, Gong S, *et al*. Neuroanatomical Alterations in Patients with Early Stage of Unilateral Pulsatile Tinnitus: a Voxel-Based Morphometry Study. *Neural Plasticity*. 2018; 2018: 1–7.
- [13] Xu Y, Shi Y, Yao J, Yang H, Ding Z, Chen QQ, *et al*. Altered brain functional connectivity and correlation with psychological status in patients with unilateral pulsatile tinnitus. *Neuroscience Letters*. 2019; 705: 235–245.
- [14] Geven LI, de Kleine E, Willemsen ATM, van Dijk P. Asymmetry in primary auditory cortex activity in tinnitus patients and controls. *Neuroscience*. 2014; 256: 117–125.
- [15] Golm D, Schmidt-Samoa C, Dechent P, Kröner-Herwig B. Neural correlates of tinnitus related distress: an fMRI-study. *Hearing Research*. 2013; 295: 87–99.
- [16] Wunderlich AP, Schönfeldt-Lecuona C, Wolf RC, Dorn K, Bachor E, Freund W. Cortical activation during a pitch discrimination task in tinnitus patients and controls—an fMRI study. *Audiology and Neurotology*. 2010; 15: 137–148.
- [17] Chen Y, Zhang J, Li X, Xia W, Feng X, Qian C, *et al*. Altered Intra- and Interregional Synchronization in Resting-State Cerebral Networks Associated with Chronic Tinnitus. *Neural Plasticity*. 2015; 2015: 1–11.
- [18] Chen Y, Xia W, Luo B, Muthaiah VPK, Xiong Z, Zhang J, *et al*. Frequency-specific alternations in the amplitude of low-frequency fluctuations in chronic tinnitus. *Frontiers in Neural Circuits*. 2015; 9: 67.
- [19] Greicius MD, Krasnow B, Reiss AL, Menon V. Functional connectivity in the resting brain: a network analysis of the default mode hypothesis. *Proceedings of the National Academy of Sciences of the United States of America*. 2003; 100: 253–258.
- [20] Schmidt SA, Akrofi K, Carpenter-Thompson JR, Husain FT. Default mode, dorsal attention and auditory resting state networks exhibit differential functional connectivity in tinnitus and hearing loss. *PLoS ONE*. 2013; 8: e76488.
- [21] Boyen K, Langers DRM, de Kleine E, van Dijk P. Gray matter in the brain: differences associated with tinnitus and hearing loss. *Hearing Research*. 2013; 295: 67–78.
- [22] Gentil A, Deverdun J, Menjot de Champfleure N, Puel J, Le Bars E, Venail F. Alterations in Regional Homogeneity in Patients with Unilateral Chronic Tinnitus. *Trends in Hearing*. 2019; 23: 2331216519830237.
- [23] Parsons LM, Petacchi A, Schmahmann JD, Bower JM. Pitch discrimination in cerebellar patients: Evidence for a sensory deficit. *Brain Research*. 2009; 1303: 84–96.
- [24] Bauer CA, Kurt W, Sybert LT, Brozoski TJ. The cerebellum as a novel tinnitus generator. *Hearing Research*. 2013; 295: 130–139.
- [25] Yang H, Zheng Y, Ou Y, Huang X. Regional homogeneity on resting state fMRI in patients with tinnitus. *Journal of Otology*. 2014; 9: 173–178.
- [26] Han L, Zhaohui L, Fei Y, Pengfei Z, Ting L, Cheng D, *et al*. Disrupted neural activity in unilateral vascular pulsatile tinnitus patients in the early stage of disease: Evidence from resting-state fMRI. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*. 2015; 59: 91–99.
- [27] Ibrahim LA, Mesik L, Ji X, Fang Q, Li H, Li Y, *et al*. Cross-Modality Sharpening of Visual Cortical Processing through Layer-1-Mediated Inhibition and Disinhibition. *Neuron*. 2016; 89: 1031–1045.
- [28] Eggermont JJ. The auditory cortex and tinnitus – a review of animal and human studies. *The European Journal of Neuroscience*. 2015; 41: 665–676.
- [29] Llinás R, Urbano FJ, Leznik E, Ramírez RR, van Marle HJF. Rhythmic and dysrhythmic thalamocortical dynamics: GABA systems and the edge effect. *Trends in Neurosciences*. 2005; 28: 325–333.
- [30] Aldhafeeri FM, Mackenzie I, Kay T, Alghamdi J, Sluming V. Neuroanatomical correlates of tinnitus revealed by cortical thickness analysis and diffusion tensor imaging. *Neuroradiology*. 2012; 54: 883–892.
- [31] Han L, Pengfei Z, Zhaohui L, Fei Y, Ting L, Cheng D, *et al*. Resting-state functional connectivity density mapping of etiology confirmed unilateral pulsatile tinnitus patients: Altered functional hubs in the early stage of disease. *Neuroscience*. 2015; 310: 27–37.
- [32] Ghaziri J, Tucholka A, Girard G, Boucher O, Houde J, Descoteaux M, *et al*. Subcortical structural connectivity of insular subregions. *Scientific Reports*. 2018; 8: 8596.