BRAIN COMMUNICATIONS

The effects of sound therapy in tinnitus are characterized by altered limbic and auditory networks

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To determine the neural mechanism underlying the effects of sound therapy on tinnitus, we hypothesize that sound therapy may be effective by modulating both local neural activity and functional connectivity that is associated with auditory perception, auditory information storage or emotional processing. In this prospective observational study, 30 tinnitus patients underwent resting-state functional magnetic resonance imaging scans at baseline and after 12 weeks of sound therapy. Thirty-two age- and gender-matched healthy controls also underwent two scans over a 12-week interval; 30 of these healthy controls were enrolled for data analysis. The amplitude of low-frequency fluctuation was analysed, and seed-based functional connectivity measures were shown to significantly alter spontaneous local brain activity and its connections to other brain regions. Interaction effects between the two groups and the two scans in local neural activity as assessed by the amplitude of low-frequency fluctuation were observed in the left parahippocampal gyrus and the right Heschl's gyrus. Importantly, local functional activity in the left parahippocampal gyrus in the patient group was significantly higher than that in the healthy controls at baseline and was reduced to relatively normal levels after treatment. Conversely, activity in the right Heschl's gyrus was significantly increased and extended beyond a relatively normal range after sound therapy. These changes were found to be positively correlated with tinnitus relief. The functional connectivity between the left parahippocampal gyrus and the cingulate cortex was higher in tinnitus patients after treatment. The alterations of local activity and functional connectivity in the left parahippocampal gyrus and right Heschl's gyrus were associated with tinnitus relief. Resting-state functional magnetic resonance imaging can provide functional information to explain and 'visualize' the mechanism underlying the effect of sound therapy on the brain.

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Abbreviations: ACC = anterior cingulate cortex; ALFF = amplitude of low-frequency fluctuation;FC = functional connectivity; FWHM = full width at half maximum; GRETNA = Graph-theoretical Network Analysis Toolkit; HC = healthy controls; MNI = Montreal Neurological Institute; ROI = regions of interest; Tf = tinnitus frequency; THI = Tinnitus Handicap Inventory.

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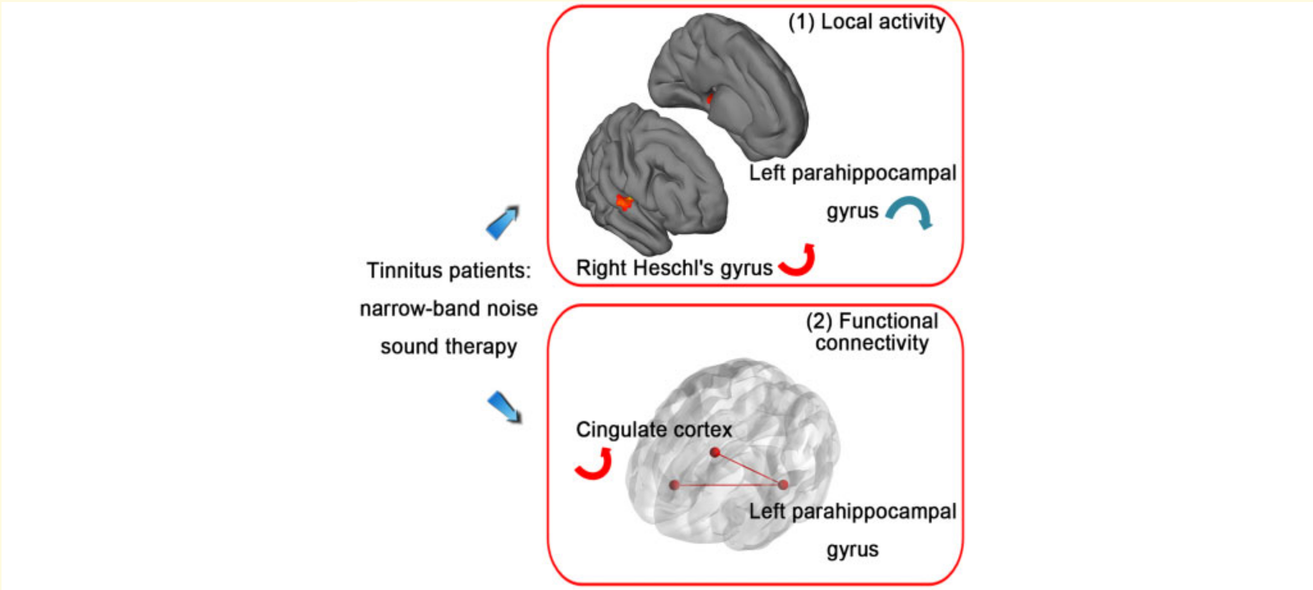

Introduction

Tinnitus is a prevalent disorder that affects ~10–25% of the population (Baguley *et al.*, 2013; Bauer, 2018). The vast majority of patients suffer from subjective tinnitus, which often involves experiencing a ringing sound in the ears without a corresponding internal or external sound source (Walker *et al.*, 2016). Persistent and bothersome tinnitus [approximately 1–3% of cases (Lanting *et al.*, 2009)] can lead to emotional distress, anxiety and sleep disturbances that significantly impair patients' quality of life.

Extensive means of treating tinnitus have been developed, which include medications (von Boetticher, 2011; Bhatt *et al.*, 2016), counselling and cognitive behaviour therapy, transcranial magnetic stimulation (Kreuzer *et al.*, 2017; Formánek *et al.*, 2018), transcranial direct current stimulation (tDCS) and transcranial magnetic stimulation (TMS) (Bae *et al.*, 2020), tinnitus retraining therapy (Scherer *et al.*, 2014; Kim *et al.*, 2016; Lee *et al.*, 2019), relaxation therapy, hearing aids (Searchfield *et al.*, 2010) and sound therapy (Hall *et al.*, 2015; Langguth, 2015; Blakley, 2016; Plein *et al.*, 2016; Makar *et al.*, 2017). According to the clinical practice guidelines of tinnitus, sound therapy is one of the recommended treatments (Tunkel *et al.*, 2014) and is used as a standard treatment in the UK (Baguley *et al.*, 2013). In terms of cost-effectiveness, sound therapy is considered to be one of the most effective methods of treatment for tinnitus patients (Aytac *et al.*, 2017). Specifically, as one of the most commonly used sound therapies, narrow-band noise is the first-line intervention method in our hospital, which is a tertiary centre subspecialized in otolaryngology in Beijing.

The purpose of sound therapy is to reduce tinnitus by increasing extrinsic sound-driven activity in the auditory system (Searchfield *et al.*, 2017). Sound therapy can induce a sense of relief from tinnitus-related distress, improving the quality of life of patients with persistent and bothersome tinnitus and enabling a significant reduction in tinnitus severity (Hobson *et al.*, 2007, 2012; Newman and Sandridge, 2012; Hoare *et al.*, 2014; Mahboubi *et al.*, 2017). The underlying mechanisms of sound treatments for tinnitus have drawn more attention and have been increasingly explored in recent years (Pienkowski, 2019). Among various kinds of efforts, resting-state functional magnetic resonance imaging is a non-invasive method that can provide insights into basic neural mechanisms by analysing blood-oxygen-level dependent signals. By using this method, different kinds of changes in neural activity after treatment have been reported, including enhanced regional activity in the precuneus/posterior cingulate cortex (Krick *et al.*, 2017*a*); greater activation of the angular gyrus (Krick *et al.*, 2017*b*); and altered functional connectivity (FC) of the thalamus (Lv *et al.*, 2020), inferior frontal gyrus and anterior cingulate cortex (ACC) (Lv *et al.*, 2019). These brain regions are closely correlated with parasympathetic control, tinnitus generation, tinnitus-cancelling systems, self-awareness or emotional regulation, which may be related to the successful treatment of tinnitus.

However, the neural basis of sound therapy remains incompletely understood. First, neural activity data have not been interpreted elaborately. Types of neural activity can be broadly divided into functional segregation and functional integration (Lv *et al.*, 2018). Functional segregation focuses on the local neural activity of specific brain regions, while functional integration focuses on the connectivity among brain areas. Analysis of each of these aspects is indispensable. However, previous studies have mainly focused on alterations in functional integration (Lv *et al.*, 2019, 2020). It is still unclear whether the neural networks that may be affected in bothersome tinnitus patients, i.e., auditory, visual, attention and control networks, would be characterized by altered local neural activity after treatment (Burton *et al.*, 2012; Wineland *et al.*, 2012). Thus, we need a reliable analytic approach to demonstrate changes in local neural activity after sound therapy using narrow-band noise. It would also be of great value to evaluate the relationship between local neural activity and its FC with other brain areas.

When analysing resting-state functional magnetic resonance imaging data, the amplitude of low-frequency fluctuation (ALFF) is a commonly used method for quantifying local brain activity with high test–retest reliability for both short-term (Kublbock *et al.*, 2014) and long-term (up to 6 months) (Zuo and Xing, 2014) follow-up data. The ALFF measures the power of the blood-oxygen-level dependent signal within the low-frequency range, reflecting the strength of neural activity (Zang *et al.*, 2007). Each voxel of the brain is analysed, thus providing a comprehensive measurement of local neural activity (Zang *et al.*, 2007). Additionally, by defining regions of interest (ROIs) from the results of regional neuroimaging studies, i.e., ALFF, we are able to analyse the relationship between brain regions. The combination of the two analytic methods can yield valid results, providing a better understanding of the neural mechanism underlying sound therapy than using either method alone.

In this prospective observational study, we analysed changes in both local neural activity and FC after treatment withnarrow-band noise. We hypothesize that sound therapy may be effective by modulating brain regions associated with auditory perception, auditory information storage or emotional processing.

Materials and methods

Standard protocol approval, registration and patient consent

This study was approved by the ethics committees of our research institution (Beijing Friendship Hospital, Capital Medical University, 2016-P2-012). Written informed consent was obtained from all study subjects.

Subjects

Thirty tinnitus patients and 32 healthy controls (HC) were enrolled in this prospective observational study. The ages, sex and education levels between patients and HC were matched. The subjects were all right handed and ranged from 18 to 65 years of age. None of the subjects suffered from hearing loss (determined by pure-tone audiometry examination, where hearing loss was defined as > 25 dB HL at frequencies from 0.25 to 8 kHz; details about the hearing thresholds are shown in Supplementary Fig. 1). According to the results of tinnitus pitch matching, tinnitus was identified as the experience of a narrow-band sound without any rhythm. Notably, all tinnitus patients were determined as recent onset, having suffered from tinnitus for less than 48 months in this study, as defined by Vanneste *et al.* (2011). Patients with hyperacusis as determined by clinicians and the Hyperacusis Questionnaire (HQ) were not included (Meeus *et al.*, 2010). Patients who underwent other treatments were excluded. Subjects with otosclerosis, Meniere's disease, head injuries, or other neurological diseases, such as stroke or sudden deafness, were not included. Furthermore, patients' scores on the Tinnitus Handicap Inventory (THI), which is one of the widely used questionnaires available for assessing the impact of tinnitus, were recorded to assess the severity of tinnitus prior to sound therapy.

Intervention (sound therapy) and clinical evaluation

For sound therapy, the intervention sound was determined through clinical examinations of the tinnitus patients, including examinations of minimum masking levels, tinnitus pitch matching patterns and loudness matching patterns. Specifically, the initial volume of the noise sound was set as the perceived loudness of tinnitus. For the frequency of the intervention sound, we first determined the tinnitus frequency according to the results of tinnitus pitch matching. We then set a narrow-band noise according to the tinnitus frequency (1 kHz narrow band i.e. tinnitus frequency ± 0.5 kHz). As such, we modified the narrow-band noise at the tinnitus pitch for each patient. The sound therapy was applied for 20 min per session, and there were three sessions per day over 12 weeks. The sound was delivered to the patients through headphones. Patients were asked to be awake when using sound therapy. For each patient, adjustments of the delivered sound parameters were made when necessary based on reports of tinnitus sensations and examinations every 2 weeks. Patients were not allowed to adjust the volume or frequency. Feedback about the treatment was collected to record the application of sound therapy every 2 weeks. Patients who did not follow the instructions were excluded from the study. None of the patients was excluded due to this criterion.

Importantly, THI scores were acquired again at the end of the treatment period (12th week). A reduction of at least 16 points in the THI score was considered effective (Zeman *et al.*, 2011).

MRI data acquisition

For each patient, to evaluate the change in brain activity under treatment, structural MRI and resting-state fMRI data were collected at baseline and at the end of therapy (12th week). The HC were also scanned at baseline as well as at the 12th week. The same scanning protocol was applied to each subject. All participants were scanned using a 3.0 T General Electric scanner with an eight-channel head coil at the Beijing Friendship Hospital, Capital Medical University, Beijing, China. High-resolution structural images were also acquired by 3D BRAVO sequencing (TR/TE/TI = 8.8/3.5/450 ms; field of view = 240 mm × 240 mm; flip angle = 15°; matrix = 256 × 256; 196 slices; 1.0 mm thickness without gap). Resting-state functional magnetic resonance imaging images were collected using a single-shot echo-planar imaging sequence (TR/TE = 2000 ms/35 ms; field of view = 240 mm × 240 mm; flip angle = 90°; matrix = 64 × 64; 33 slices; 3.0 mm slice thickness, 1.0 mm gap).

Image preprocessing

Image preprocessing was performed using Statistical Parametric Mapping 12 (http://www.fil.ion.ucl.ac.uk/spm) and the Graph-theoretical Network Analysis Toolkit (GRETNA; http://www.nitrc.org/projects/gretna/) toolbox (Wang *et al.*, 2015). The preprocessing procedures are described in more detail in previous articles (Lv *et al.*, 2018). In brief, procedures involved discarding the first 10 functional volumes (the first 20 s of fMRI data), slice-timing, head motion correction and normalization to Montreal Neurological Institute (MNI) space by applying the transformation parameters obtained from the high-resolution structural images using the Diffeomorphic Anatomical Registration Through the Exponentiated Lie algebra technique (Ashburner, 2007). The resampling voxel size was set to 3.0 mm × 3.0 mm × 3.0 mm voxels. Two healthy subjects were excluded based on the correction exclusion criteria (spatial movement in any direction of more than 2.0 mm or 2°). The subjects exhibited no significant group differences in terms of head motion parameters. Then, qualified images were smoothed using a 4.0-mm full width at half maximum (FWHM) Gaussian kernel, followed by detrending, nuisance covariate regressing [global signal, white matter signal, cerebrospinal fluid signal and Friston 24 head motion parameters as indicated by Yan *et al.* (2013)], and band-pass filtering (0.01–0.08 Hz) for each voxel. The global/cerebrospinal fluid signal/white matter signal was calculated by using the whole brain, cerebrospinal fluid signal and white matter masks in the standard MNI space based on the template from Statistical Parametric Mapping 12.

ALFF/FC calculations

Altered local neural activity after sound therapy determined by ALFF values

We first identified whether local neural activity was altered with sound therapy. After image preprocessing, whole-brain voxelwise ALFFs for each subject were calculated with qualified images. For standardization purposes, the subject-level voxelwise ALFF map was converted into a z-score map for further statistical analysis.

To determine the group × time interaction effect between the two groups and the two scans, the main effects of group (the tinnitus patient and healthy control groups) and time (baseline and 12-week follow-up period), two-way mixed model analysis of variance (ANOVA) and *post hoc* analyses were performed (the design matrix is shown in Supplementary Fig. 2).

Altered FC after treatment determined by FC analysis

Head motion has been reported as a potential factor that could affect the results of resting-state fMRI (Parkes *et al.*, 2017), especially the FC results (Power *et al.*, 2012, 2014; Van Dijk *et al.*, 2012). To further validate the FC results, we conservatively evaluated the head motion effect with the 'scrubbing' method in the GRETNA toolbox (Wang *et al.*, 2015). This step might further reduce the bias on the resting-state fMRI signal induced by head motion artefacts. In brief, we first calculated the framewise displacement between the neighbouring volumes within each subject. Volumes with a framewise displacement above 0.5 mm were scrubbed with their adjacent volumes (previous one time point and subsequent two timepoints). Then, the resultant scrubbed data were used for subsequent FC map calculation and statistical analysis.

Furthermore, to examine whether levels of FC between local brain regions and the whole brain would be altered after sound therapy in tinnitus patients, we performed a seed-based FC analysis. Brain regions presenting significant ALFF interactions were selected as seeds. FC analyses were conducted using REST (http://www.restfmri.net) software (Song *et al.*, 2011). The calculation method used is in line with those of previous studies (Lv *et al.*, 2016, 2018). We also applied Fisher's z-transform to improve the normality of the correlation coefficients (Lowe *et al.*, 1998).

Statistical analysis

Demographic data were compared through two-sample *t*-tests, paired two-sample *t*-tests and Fisher's exact test using SPSS 12.0 software (SPSS, Inc., Chicago, IL, USA). *P*-values <0.05 were considered statistically significant. Longitudinal changes in the THI score were also analysed by using paired two-sample *t*-tests.

For the results of ALFF/FC, the corrected $P < 0.05$ was used as the threshold determined by the Monte Carlo simulation [uncorrected $P < 0.001$ and minimum 23 voxels in a cluster, estimated FWHMx = 7.39 mm, FWHMy = 7.34 mm, FWHMz = 7.80 mm, 1000 simulations, for ALFF calculation and uncorrected $P < 0.001$ and minimum 22 voxels in a cluster, estimated FWHMx = 7.36 mm, FWHMy = 7.45 mm, FWHMz = 7.81 mm, 1000 simulations, for FC calculation, family-wise error-corrected on cluster level (Eklund *et al.*, 2016)].

To investigate the relationship between ALFF/FC and clinical variables, correlation analyses were conducted on changes (baseline minus 12th week) observed in the THI scores and on functional activity measurements (i.e. ALFF and seed-based FC) taken from brain regions with a significant interaction effect. We also analysed the correlation between the per cent improvement of ALFF/FC and that of the THI score. Bonferroni correction was applied to account for multiple testing with age, gender and education levels as covariates. The results were visualized with the BrainNet Viewer and REST Slice Viewer (http://www.nitrc.org/projects/bnv/) (Song *et al.*, 2011; Xia *et al.*, 2013).

Data availability statement

Anonymized data not published within this article will be made available by request from any qualified investigator.

Results

Sample characteristics

There were no significant differences between the tinnitus patients and HC in terms of age, gender or education level (Table 1). After sound therapy, the total THI scores decreased significantly.

Altered local neural activity after sound therapy determined by ALFF values

We observed a significant main effect of group on ALFF in the bilateral parahippocampal gyrus, left inferior frontal gyrus, left caudate nucleus, left middle temporal gyrus, right inferior parietal lobule and left precuneus (tinnitus patients > HC, Supplementary Table 1, Supplementary Fig. 3). No regions showed significant main effects of time.

As shown in Table 2 and Fig. 1, significant group × time interaction effects between the two groups and two scans on ALFF values were observed in the left parahippocampal gyrus and right Heschl's gyrus, the key region of the primary auditory cortex. A *post hoc* analysis showed that the interaction effects observed in the left parahippocampal gyrus were driven by ALFF values that decreased in the tinnitus group after treatment, while a stable ALFF was observed in the control group ($P < 0.05$, corrected). Importantly, the ALFF values of the tinnitus patients observed after therapy were similar to those of the HC (at baseline and 12 weeks of follow-up), suggesting that local functional activity in the left parahippocampal gyrus approached relatively normal levels after sound therapy.

For the right Heschl's gyrus, interaction effects between the two groups and two scans were driven by significantly increased ALFF values in the tinnitus group after treatment ($P < 0.05$, corrected) compared to those of the tinnitus patients at baseline and to those of the HC. This suggests that increased levels of local neural activity in the right Heschl's gyrus in the patient group extended beyond a relatively normal range after treatment.

Interaction effect on functional connectivities of the left parahippocampal gyrus and the right Heschl's gyrus after sound therapy

The left parahippocampal gyrus was set as a seed region to perform seed-based FC analysis. We observed a significant main effect of group on the z-values of the FCs between the left parahippocampal gyrus and the bilateral fusiform gyrus, calcarine sulcus and right caudate nucleus (tinnitus patients > HC, Fig. 2A). No regions showed significant main effects of time. As shown in Table 2 and

Table 1. Summarized demographic and clinical characteristics of the enrolled subjects

	Tinnitus patients (baseline, *n* = 30)	Tinnitus patients (12th week, *n* = 30)	Healthy controls (baseline, *n* = 30)	Healthy controls (12th week, *n* = 30)	*P*-value
Age (years)	21–62 (37.3 ± 10.7)		25–59 (39.1 ± 11.0)		0.33[a]
Gender (male/female)	14/16		14/16		>0.99[b]
Education (years)	6–16 (11.4 ± 2.9)		6–19 (12.2 ± 3.6)		0.20[a]
Handedness	30 right handed		30 right handed		>0.99[a]
Tinnitus duration (months)	6–48 (24.5 ± 13.0)		NA		NA
THI score	40–90 (63.3 ± 14.8)	12–50 (30.7 ± 11.2)	NA		<0.01[c]
△THI score	20–52 (32.5 ± 9.3)		NA		NA

Data are presented as min–max ranges (mean ± SD) but not in the case of gender.

△THI score = longitudinal changes in THI scores, i.e., THI score for baseline minus the 12th week; NA = not applicable; THI = Tinnitus Handicap Inventory.

[a]Two-sample *t*-tests.

[b]Chi-square test.

[c]Paired sample *t*-tests.

Table 2. Brain regions showing significant group × time interaction effects on local neural activity reflected by altered ALFF values, and on z-values of FC with the left parahippocampal gyrus

	Brain region	Peak MNI, mm			*F*-score	Voxels
		x	*y*	*z*		
ALFF	Left parahippocampal gyrus	−33	−36	−9	7.45	33
	Right superior temporal gyrus	54	−12	9	8.09	23
FC	Left middle cingulate cortex	−3	6	30	6.47	27
	Right ACC	9	36	6	14.31	25

ALFF = amplitude of low-frequency fluctuation; FC = functional connectivity; MNI = Montreal Neurological Institute.
The threshold was set to $P < 0.05$ (corrected).

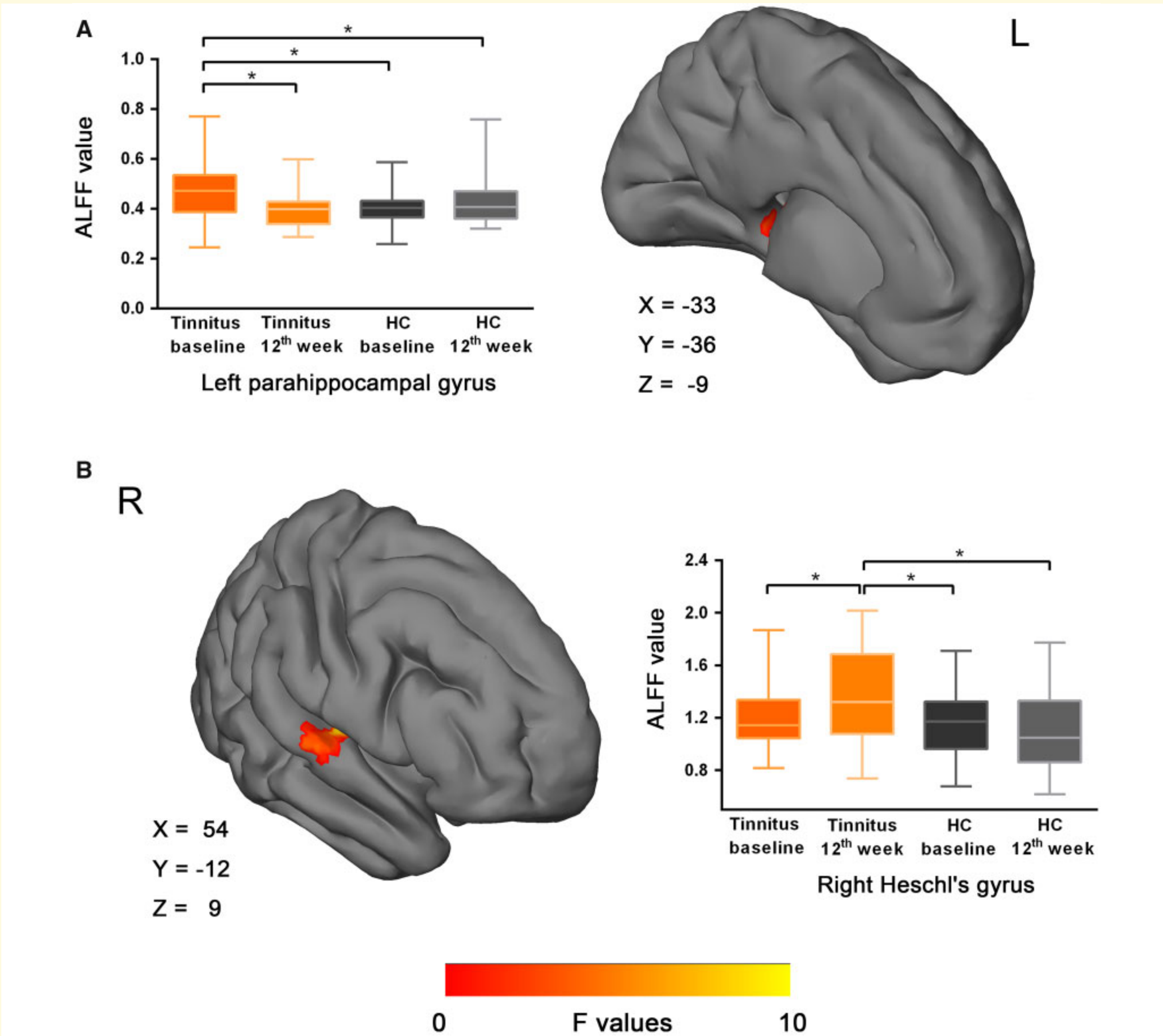

Figure 1. Significant group × time interaction effects on ALFF values. The results were observed in the left parahippocampal gyrus and right Heschl's gyrus. A *post hoc* analysis and bar maps identify the source of these effects. (**A**) For the left parahippocampal gyrus, the interaction effect between the two groups and two scans was driven by restored ALFF values in the tinnitus group after sound therapy, while a stable ALFF was observed in the control group. (**B**) For the right Heschl's gyrus, the interaction effect was driven by increased ALFF values observed in tinnitus patients after treatment, while there was no significant change in the healthy control group. X, Y and Z denote peak F values in the MNI (Montreal Neurological Institute) space. HC = healthy controls.

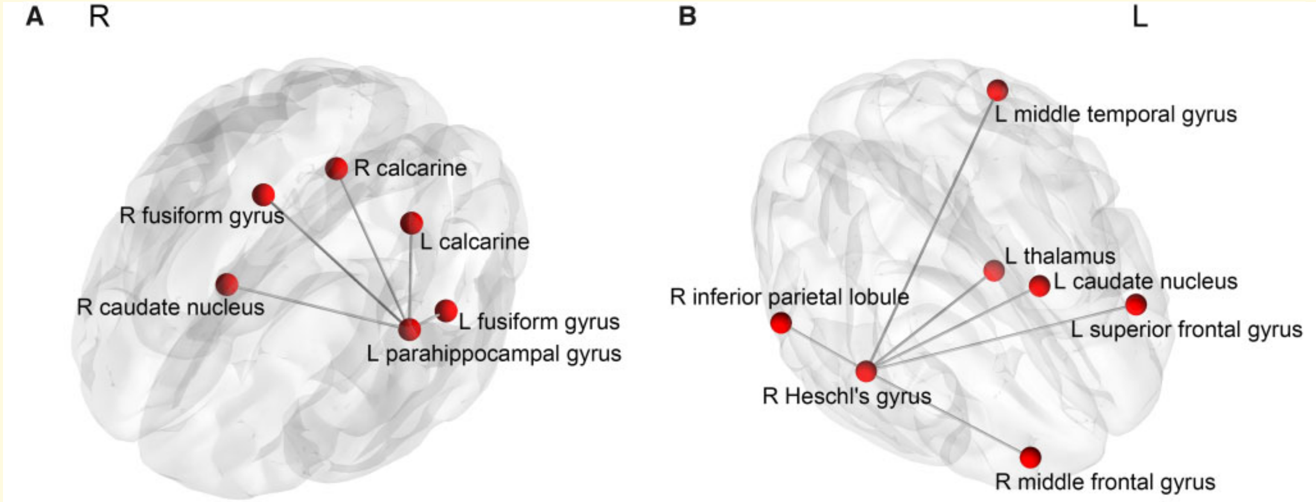

Figure 2. Significant main effect of group on the z-values of FC. We observed a significant main effect of group on the z-values of the FC between the left parahippocampal gyrus and bilateral fusiform gyrus, calcarine sulcus and right caudate nucleus. A significant main effect of group on the z-values of the FC between the right Heschl's gyrus and left thalamus, left middle temporal gyrus, right middle frontal gyrus, right inferior parietal lobule, left caudate nucleus and left superior frontal gyrus was observed. L = left; R = right.

Fig. 3, a significant group × time interaction effect on the z-values of the FC was observed between the left parahippocampal gyrus and the right ACC and between the left parahippocampal gyrus and the left middle cingulate cortex after corrections. Increased levels of FC were found to be significant at the 12th week in the patient group, which exceeded the relatively normal range after sound therapy. The functional connections remained stable in the healthy subjects.

When the right Heschl's gyrus was used as the seed region, a significant main effect of group on the z-values of the FCs between the right Heschl's gyrus and the left thalamus, left middle temporal gyrus, right middle frontal gyrus, right inferior parietal lobule, left caudate nucleus and left superior frontal gyrus was observed (tinnitus patients > HC, Fig. 2B). No regions showed significant main effects of time. No significant interaction effect remained after multiple comparison corrections were made according to FC maps.

Relationship between ALFF/FC and clinical variables

Significant positive correlations between the decreased ALFF value of the left parahippocampal gyrus and the △THI score were observed ($r = 0.434$, $P = 0.016$) after the effects of age, gender and disease duration were removed (Bonferroni corrected $P < 0.05/2 = 0.025$). Positive correlations between the increased ALFF value observed in the right Heschl's gyrus and the △THI score were identified ($r = 0.385$, $P = 0.035$) with age, gender and disease duration used as covariates but were not statistically significant after Bonferroni correction for multiple comparisons ($P < 0.05/2 = 0.025$). No additional significant correlation was found.

Discussion

Sound therapy aims to desensitize the perception of tinnitus rather than eliminate tinnitus symptoms. This idea is supported by the fact that only a limited number of brain regions were affected by the intervention. According to a meta-analysis (Chen *et al.*, 2017), several brain regions with abnormal local neural activity in patients with tinnitus are commonly reported. Among those brain areas, only neural activity in the left parahippocampus was identified to be decreased after therapy in this study.

The neural functions of the parahippocampal gyrus are regarded to be critical to the emotional processing of tinnitus. As one of the important brain regions belonging to the limbic network, numerous results note the involvement of the parahippocampal gyrus in the perceptions of tinnitus (Maudoux *et al.*, 2012a, b; Ridder *et al.*, 2014; Leaver *et al.*, 2016; Vanneste and De, 2016). When processing external effective sounds, the bilateral parahippocampal gyri are more engaged in mild tinnitus patients than in HC, especially in the left brain region (Carpenter-Thompson *et al.*, 2014).

In addition, the parahippocampal gyrus was also considered to be associated with auditory information storage. It may serve as the source of phantom auditory perception by supplying missing auditory information, according to the Bayesian brain theory of tinnitus generation (Vanneste *et al.*, 2018; Han *et al.*, 2020). In this study, the enrolled tinnitus patients had normal hearing

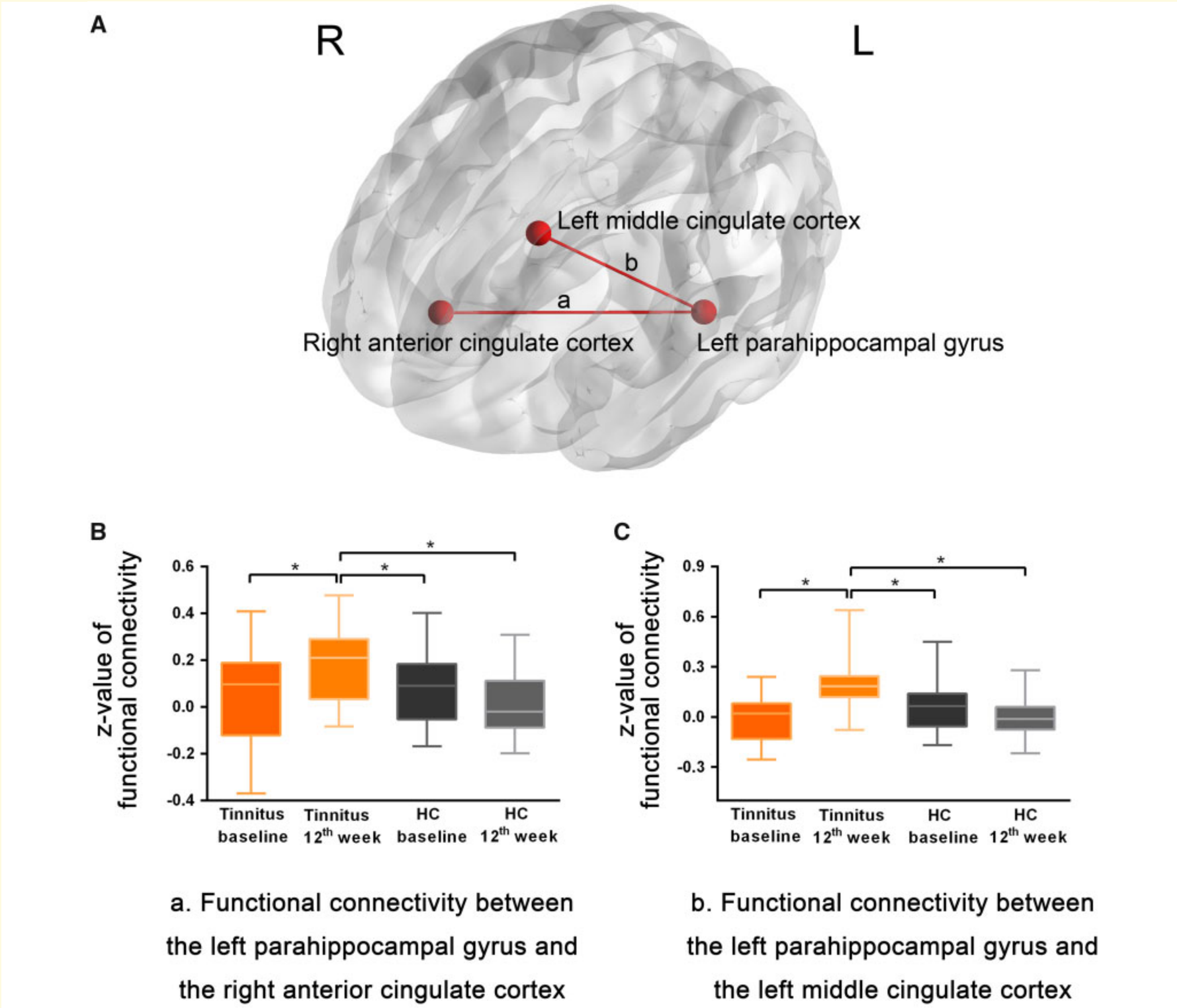

Figure 3. Significant group × time interaction effect on the FC of the left parahippocampal gyrus. (**A**) A significant group × time interaction effect was observed on the z-values of the FC between the left parahippocampal gyrus and the right ACC and left middle cingulate cortex after corrections were made for multiple comparisons. (**B, C**) Significantly increased levels of FC were observed in tinnitus patients after treatment, while functional connections remained stable in healthy subjects. HC = healthy controls.

thresholds up to 8 kHz. However, they might have lost a small amount of hearing, especially in the high-frequency region, which might have resulted in the generation of tinnitus in the patient group due to the unnecessary compensatory activity of the parahippocampal gyrus.

The decreased activity in the parahippocampus may be associated with less emotional processing of the enrolled tinnitus patients in this study. It may also be regarded as normalization of the unnecessary compensatory activity of the parahippocampal gyrus due to the sound-enriched environment in the patient group. We suggest that local neural activity of the parahippocampal gyrus may serve as an indicator of clinical responses to sound therapy. An Auditory Brainstem Response test may be helpful for tinnitus patients to detect possible hearing impairment in further studies.

Increased connections between the parahippocampal gyrus and the auditory cortex and dorsal attention network were identified in tinnitus patients in previous studies (Vanneste *et al.*, 2011; Maudoux *et al.*, 2012*a*, *b*; Schmidt *et al.*, 2013). However, we did not find decreased FC among the parahippocampal gyrus and other brain regions. Patients who underwent sound therapy were not considered to be 'healthy subjects'. Thus, not all of the neural abnormalities were found to be altered, which may explain why the stability and reliability of ALFF was considered higher than seed-based FC

(Zuo and Xing, 2014). Further studies are still needed to validate changes in FC after treatment.

As core structures of the limbic system, the involvement of the ACC and middle cingulate cortex may not be specific to tinnitus (Golm *et al.*, 2013). Combined with other brain regions, such as the insula and precuneus, these cortexes form a general distress network that can facilitate interactions between the limbic and auditory networks. Impaired FC between the left parahippocampal gyrus and the limbic system has also been identified in tinnitus groups in previous studies (Leaver *et al.*, 2016). When tinnitus is suppressed for a short period after narrow-band noise stimulation, ACC activity levels are enhanced (Mirz *et al.*, 2000). Therapy-specific changes are also characterized by enhanced levels of activity in the cingulate cortex, as reported by Krick *et al.* (2017*a*). Enhanced FC values observed between the parahippocampal gyrus and cingulate cortex may serve as neural feedback routes within the limbic system. The abnormal activity of ACC may also be associated with thalamocortical dysrhythmia, which was reported by a recent study that analysed by electroencephalography (Vanneste *et al.*, 2018).

There is a worldwide debate on the central neural gain and discussing the signal modification of the auditory system (Sedley, 2019). Recent data have provided evidence that there is a lack of central neural gain in tinnitus patients (Hofmeier *et al.*, 2018). Thus, Benedikt *et al.* suggested that neural correlates of tinnitus may need advanced sound stimulation, which was the theoretical basis for the application of sound generator-based therapies. Our findings of rising activity in Heschl's gyrus after sound therapy may be a valuable contribution to support the theory of increased central neural gain in the auditory system after treatment. Furthermore, a previous study also reported the increased grey matter in the same brain region, the right Heschl's gyrus, in tinnitus patients after treatment (Krick *et al.*, 2015), additionally supporting this theory from an anatomical point of view. Consequently, to monitor the changes in neural activity, the ALFF values may lead to a suitable model of explanation.

Several issues need to be further addressed. First, ALFF is a widely used analytic method. However, the concept that ALFF reflects local brain activity may not be universally accepted. Additionally, FC measurements are based on the selection of ROIs. In this study, only two brain regions were selected as seeds according to the results of voxelwise ALFF calculation, potentially explaining the limited number of significantly altered FCs observed. Second, altered FC values observed within and between neural networks should be further discussed. More studies using different analytical methods may explore the effect of sound therapy on different factors. Third, the tinnitus patients enrolled in this study did not suffer from hearing loss, which is not representative of most patients with tinnitus. Additional ABR tests may better characterize hearing impairment. It may be more helpful to analyse non-responders to better characterize the mechanism of sound therapy. IT may also be helpful to analyse patients with a wider range of THI scores or patients whose disease severity was evaluated by other methods [e.g. the visual analogue scale, tinnitus loudness, the tinnitus functional index (Lewis *et al.*, 2020)]. Patients with sham treatment may be needed to account for the placebo effect of sound therapy.

In conclusion, we proved our hypothesis that sound therapy is effective by modulating both local neural activity and FC, which is associated with auditory perception, auditory information storage or emotional processing. The alterations of local activity and FC in the left parahippocampal gyrus and right Heschl's gyrus were associated with tinnitus relief. Increased levels of FC between the left parahippocampal gyrus and cingulate cortex may serve as neural feedback routes within the limbic system.

Supplementary material

Supplementary material is available at *Brain Communications* online.

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Conflict of interest

The authors report no conflict of interest.

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