An Exploratory Clinical Study on fMRI-Based Evaluation of Intervention Targets for Auricular Acupuncture Therapy

Study Protocol

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一、Study Background

Auricular Vagus Nerve Stimulation (aVNS) regulates central nervous system function and influences neural activity in the brain by stimulating the vagus nerve branch in the concha region, showing broad application prospects in the field of neuropsychiatric diseases in recent years [1]. The intervention methods for aVNS include electrical stimulation of the transcutaneous auricular vagus nerve and stimulation of the auricular acupoint vagus nerve. Auricular acupuncture, a microsystem therapy, is theoretically based on the reflective correspondence between specific auricular points and system-level functional areas. This method simulates the effects of conventional invasive vagus nerve stimulation by applying needles to the auricle, thereby providing new avenues for treating conditions such as epilepsy, depression, pain, and cardiac diseases. The existing literature shows that auricular acupuncture vagus nerve stimulation has been investigated in several diseases, demonstrating its efficacy for depression [2], migraine [3, 4], and other conditions. Clinically, auricular acupuncture has proven to be significantly effective for anxiety and pain. Similar to body acupuncture, auricular acupuncture reduces preoperative anxiety, indicating comparable efficacy to traditional body acupuncture in anxiety relief [5]. Furthermore, auricular acupuncture has an analgesic effect on acute pain (such as sore throat), reducing pain scores and the need for analgesic medications [6]. In temporomandibular joint pain, auricular acupuncture, as an adjuvant therapy, can synergistically enhance the effects of conventional treatment, further supporting its ability to modulate pain pathways [7]. As anxiety and pain are common triggers of sleep disorders, auricular acupuncture may indirectly improve sleep quality by alleviating anxiety. These improvements may be related to the upregulation of plasma Brain-Derived Neurotrophic Factor (BDNF) levels [8]. Although these findings confirm the clinical efficacy of aVNS, the central nervous system mechanism of action has not been fully elucidated.

Studies suggest that aVNS may affect brain function through multiple pathways, including the modulation of neurotransmitter release, neurotrophic factor expression, and neuroinflammatory responses [8]. In particular, BDNF, as an important neurotrophic factor, is not only related to the efficacy of aVNS but may also participate in neuroplastic processes, affecting brain network functional reorganization. However, current research on how aVNS modulates the functional connectivity of specific brain networks—especially neural networks closely related to cognition and emotion regulation, such as those involving the insula and medial prefrontal cortex—remains insufficient.

Resting-state functional magnetic resonance imaging (rs-fMRI) provides a key tool for investigating the modulation effects of Vagus Nerve Stimulation (VNS) on brain functional connectivity (FC) by measuring the blood-oxygen-level–dependent (BOLD) signal arising from spontaneous neural activity in the absence of tasks. Functional connectivity (FC), which refers to temporal correlations in spontaneous neural activity between distinct brain regions, is known to be aberrant in various neurological disorders [8-9]. Specific studies have demonstrated that aVNS can significantly modulate FC in multiple brain networks. Studies have indicated that aVNS attenuates negative FC between the insula and the medial prefrontal cortex (mPFC), a mechanism that may enhance the balance between interoceptive awareness and cognitive experience [1]. In patients with primary insomnia, rs-fMRI analyses revealed that taVNS reduced FC between the mPFC and dorsal anterior cingulate cortex (dACC, part of the salience network), as well as between the mPFC and occipital cortex. This reduction in FC was positively correlated with improvements in the Pittsburgh Sleep Quality Index (PSQI) scores, suggesting that taVNS may improve sleep by modulating interactions between the default mode network (DMN), salience network, and the visual cortex [8]. Furthermore, in migraine research, repeated taVNS treatment modulated resting-state FC (rsFC) between brainstem areas (e.g., NTS, RN), the limbic system (bilateral hippocampus), pain-processing regions (bilateral postcentral gyri, thalamus, and mPFC), and basal ganglia (putamen/caudate). Notably, changes in rsFC between the RN and putamen were significantly correlated with a reduction in monthly migraine days, underscoring the role of taVNS in pain modulation pathways [9]. Another study on primary insomnia found that taVNS reduced FC between the basal forebrain (BF) and the visual cortex (e.g., middle occipital gyrus, fusiform gyrus), sensorimotor cortex (supplementary motor area), and mPFC. Weakening of the connection between the basal forebrain and middle occipital gyrus was associated with improvements in the Insomnia Severity Index (ISI). More importantly, baseline FC within the basal-forebrain–visual circuit predicts therapeutic response to taVNS, supporting its potential as a biomarker for personalized treatment [10].Recent research has shown that transcranial auricular vagus nerve stimulation (taVNS) effectively reduces negative functional connectivity between the insula and the medial prefrontal cortex (MPFC)[1]. This modulation of neural circuitry is linked to improvements in both proprioceptive awareness and emotional cognition, offering key insights into the therapeutic mechanisms of taVNS.

This study systematically investigates whether auricular acupuncture elicits neuromodulatory effects comparable to taVNS on functional connectivity between the insula and the medial prefrontal cortex (mPFC) in healthy subjects using resting-state functional magnetic resonance imaging (rs-fMRI). By characterizing the baseline neurophysiological effects of auricular acupuncture, the work clarifies its core regulatory mechanisms in cognition and mood. These findings establish key theoretical and evaluative frameworks for subsequent clinical translation in insomnia, depression, and related disorders.

二、Study Objectives

Primary Objective: This study investigates whether auricular acupuncture therapy can modulate the functional connectivity between the insula and medial prefrontal cortex (MPFC) via resting-state functional magnetic resonance imaging (rs-fMRI), similar to transcranial vagus nerve stimulation (taVNS), and analyzes its association with improvements in proprioceptive function.

Mechanistic Objective: To investigate how aVNS influences the integration of interoceptive processing (bodily signal processing) and cognitive function (cerebral cognitive processing) by examining its regulatory impact on this specific neural circuit.

三、Study Design

3.1 Design Type

This was a parallel-group, randomized, sham-controlled clinical trial.

The study was conducted in accordance with the Consolidated Standards of Reporting Trials (CONSORT) guidelines and the Standards for Reporting Interventions guidelines. All participants provided written informed consent prior to participation and received incentives or compensation for participating in the study.

3.2 Study Population and Recruitment

Healthy adults were recruited from Taizhou People's Hospital through hospital outpatient clinics and promotional posters on the hospital's official WeChat accounts. The study adhered to the principle of voluntary participation, and all subjects were required to sign an informed consent form before enrollment. All participants were assessed and screened by a physician.

3.3 Sample Size

The sample size estimation for functional MRI (fMRI) studies was based on Desmond's research findings. When analyzing fMRI data using a liberal threshold of 0.05, at least 12 subjects are required to achieve 80% or higher activation intensity at the single-voxel level [11]. This study randomly recruited 32 subjects, with 16 subjects per group, to systematically analyze changes in brain function before and after auricular acupuncture intervention, combined with its efficacy.

3.4 Study Population and Criteria

Study Subjects

Inclusion Criteria:

（1）Age 18–30 years;

（2）Basically normal diet and sleep;

（3）No history of mental illness;

（4）No MRI contraindications (e.g., metal implants or pacemakers) or claustrophobia；

（5）Willing to participate in this study and sign the informed consent form.

Exclusion Criteria:

（1）Presence of auricular skin lesions or allergy to adhesive ear patches;

（2）Currently receiving regular acupuncture treatment;

（3）History of bleeding disorders or anticoagulant use (increased bleeding risk);

（4）Previous history of syncope during acupuncture.

3.5 Randomization and Blinding

The enrolled 32 subjects were randomly assigned in a 1:1 ratio to two groups: the real auricular acupuncture vagus nerve stimulation group (AA) and the sham auricular acupuncture control group (SA).

This study employed a double-blind design, meaning that neither the subjects in each group nor the acupuncturist knew whether they belonged to the AA group or the SA group.

The sham stimulation group used press needles without tips at the same acupoints, preventing the subjects from distinguishing whether they were receiving real or sham stimulation, thereby reducing expectancy effects. Randomization and allocation concealment were performed by an independent statistician, ensuring that neither the study participants nor the researchers could influence the assignment. The success of participant blinding was assessed using the Bang Blinding Index (BBI); blinding was considered successful when the BBI value was between -0.2 and 0.2.

3.6 Interventions

3.6.1Auricular Press Needle Acupuncture

Auricular Points: Heart, Kidney, Shenmen, Subcortex

①Before the application, the participants rested for 5 min, followed by baseline fMRI examination (including T1-weighted and T2-weighted images).

②After the examination, auricular press needles were inserted. The physician's hands and local acupoint areas were routinely disinfected. The experimental group used Dingshi brand disposable sterile press needles (with release paper, Medical Device Registration No.: Su Xie Zhu Zhun 20212200040, specification 0.20×1.3 mm). Disposable, sterile press needles without tips were used in the control group. The needle body was inserted at the aforementioned points, the release paper was removed, and the adhesive tape was gently pressed to ensure tight adhesion. Both ears were treated at each time-point.

③After application, each acupoint was pressed 20 times. Pressing all eight points twice took approximately 2 min to complete. Rest for 10 min. A second pressing session was performed for 2 min. Rest for 10 min. A third pressing session was performed for 2 min, and the press needles were removed. (Total duration: approximately 26 min).

④Immediately an fMRI examination (T2-weighted images only) was performed to observe changes in brain regions.

The intervention involved applying auricular press needles (a type of intradermal embedding needle) to specific acupoints.

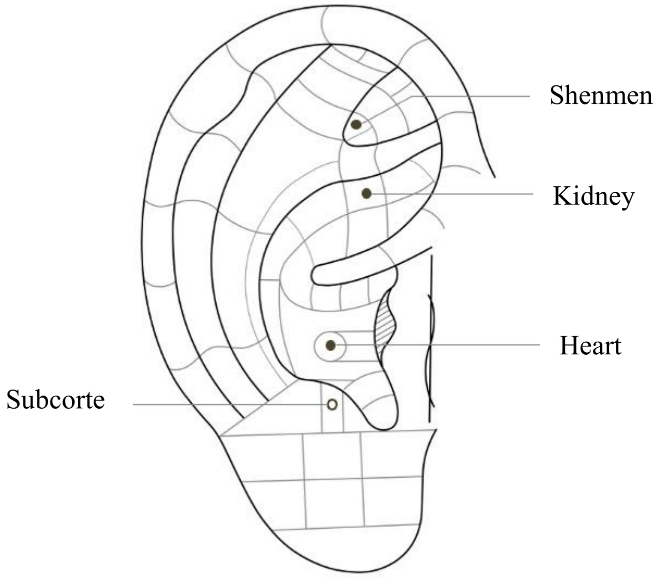

Figure 1. Localization of selected auricular points.

Table 1. Common names, locations, and indications of auricular points.

Common Name	**Acupuncture Point**	**Anatomical Location**	**Indications Based on TCM and Scientific Research**	**Mental Health and Depression Indications**
Shenmen	TF4	The upper portion of the apex of the triangular fossa iscloser to the upper branch of the antihelix.	Analgesic and anti inflammatory action is the most critical anesthesia point on the ear.	Sedative point. Calms the mind.
Heart	CO15	In the center of the cavum conchae	Used for cardiac complaints such as angina, cardiac rhythm disorders	Indicated for anxiety, depression, distress, mental agitation, and insomnia.
Kidney	CO10	In the cymba conchae, in a groove below the start of the inferior crus.	Used for issues in the urinary and genital systems. Assists with tinnitus, auditory conditions, and sleep disorders.	Indicated for fear, insecurity, and lack of willpower.
Subcortex	AT4	Inner and anterior faces of the antitragus	Rebalances nervous, digestive, and cardiovascular functions	Harmonizes cortical excitation and inhibition

3.6.2 ECG Patch

The ECG patch operation was divided into three stages: from the start of auricular press needle treatment to the end of the second resting-state fMRI scan.

Operation method:

①Baseline ECG data collection: After the subject rested for 5 min, the patch was attached to the corresponding chest wall position for 5 min of ECG monitoring, after which the ECG patch was removed.

②Treatment-period ECG data collection: After the baseline fMRI examination, the ECG patch was attached. Monitoring continued throughout the auricular acupuncture treatment period. The ECG patch was removed after treatment completion.

③Post-treatment ECG data collection: After the second fMRI examination, the ECG patch was attached, 5 min of ECG monitoring was performed, and then the ECG patch was removed.

The ECG data recorded by the device were transmitted to a computer or cloud, automatically analyzed using system software, or reviewed by a physician.

3.6.3 Imaging Examination

All participants underwent magnetic resonance imaging on the same day as the auricular acupuncture treatment using a Siemens Skyra 3T scanner equipped with a 16-channel head coil. The imaging protocols included conventional T1-weighted imaging (T1WI), T2-weighted imaging (T2WI), T2-fluid-attenuated inversion recovery (T2-FLAIR), high-resolution 3D-T1WI, and resting-state functional MRI (rs-fMRI). Prior to scanning, the participants were fitted with noise-canceling headphones and placed in a supine position. Head motion was minimized using foam padding. Participants were instructed to keep their eyes closed, maintain a wakeful state, and avoid engaging in any specific cognitive tasks. High-resolution T1-weighted structural scanning parameters were as follows: TR = 2300 ms, TE = 2.98 ms, FA = 9 degrees, FOV = 256 mm × 256 mm, voxel size = 1 mm × 1 mm × 1 mm, slice number = 176, thickness = 1 mm; for rs-fMRI, TR = 2160 ms, TE = 30 ms, FA = 90 degrees, FOV = 256 mm × 256 mm, matrix = 64 × 64, number of excitations (NEX) = 1, voxel size = 4 mm × 4 mm × 3 mm, thickness = 3 mm, slice gap = 0.75 mm, 40 slices, total acquisition time = 518 s.

3.7 Observational Indicators

Resting-state functional magnetic resonance imaging was used to conduct pre- and post-intervention comparative analyses in participants receiving auricular acupuncture.

Data collection is divided into two stages:

Baseline: Collection of demographic data, baseline fMRI scan, and clinical assessment (T0).

post-intervention:fMRI scan and clinical assessment immediately after the intervention (T1).

Primary Outcome Measure

Functional connectivity strength between the insula and the medial prefrontal cortex (MPFC)(T1-T0)

This connectivity strength serves as a proxy for the efficiency of information exchange between the “interoceptive hub” and “the self-referential/emotion-regulatory hub”. An increase following auricular acupuncture may reflect enhanced psychosomatic integration and top-down emotional regulation, suggesting a functional optimization of this circuit. Conversely, a decrease could indicate a disruption of pathological hyper-connectivity related to emotional constraint or an upregulation of control—mechanisms consistent with the observed alleviation of emotional symptoms

Secondary Outcome Measures

①Local brain activity indicators:regional homogeneity (ReHo), amplitude of low-frequency fluctuation (ALFF), fractional ALFF (fALFF)(T1-T0);

ReHo captures the temporal synchrony of neural activity within local brain regions. Following auricular acupuncture, increased ReHo in regions such as the insula and prefrontal cortex may reflect improved neural coordination and functional integration. Conversely, decreased ReHo in specific areas could indicate a modulatory effect on abnormally elevated local synchrony.

ALFF reflects the intensity of spontaneous neural activity in the brain. An increase in ALFF within emotion- and cognition-related regions following auricular acupuncture may indicate an elevation in baseline neural activity or metabolic activity in these areas. Conversely, a decrease in ALFF in regions such as the limbic system could reflect an inhibitory modulatory effect of the intervention on hyperactivity, potentially contributing to emotional stabilization.

fALFF: Changes in fALFF following the intervention allow a more specific assessment of auricular acupuncture's regulatory effects on intrinsic neural oscillations related to cognition and emotion. When interpreted in conjunction with ALFF changes, the direction of fALFF alteration can help elucidate the specific mechanisms through which auricular acupuncture modulates neural activity patterns.

②Interhemispheric coordination index: voxel-mirrored homotopic connectivity (VMHC)

The VMHC value quantifies the functional coordination between homotopic regions across the two cerebral hemispheres. Following auricular acupuncture, enhanced VMHC in regions associated with emotional regulation and cognitive control, such as the prefrontal cortex and anterior cingulate cortex, may suggest improved interhemispheric balance and integration of emotional processing. Conversely, changes in VMHC within specific regions could help clarify how the intervention modulates functional imbalances between hemispheres, particularly those related to constrained emotional states.

3.8Functional Image Processing and Statistical Analysis

3.8.1 Resting-state fMRI Data Processing

All functional imaging data were processed and analyzed using MATLAB R2022b (MathWorks) with the SPM12 toolkit, RESTplus v1.25, and DPABI v7.0 software packages. The preprocessing steps included: ①data format conversion; ②removal of the first 10 time points to eliminate unstable initial signals; ③head motion realignment and slice-timing correction. Individuals with head movements exceeding 2 mm or 2 degrees were removed; ④spatial normalization to the Montreal Neurological Institute template using the DARTEL registration algorithm, followed by resampling to an isotropic voxel size of 3 mm × 3 mm × 3 mm；⑤spatial smoothing using a 6-mm Gaussian kernel; ⑥linear detrending; ⑦regression of nuisance covariates, including 24-head motion parameters, white-matter signal, and cerebrospinal fluid signal; ⑧application of a 0.01 to 0.08 Hz bandpass filter.

Mean Regional Homogeneity (mReHo): ReHo maps were generated by calculating

Kendall's coefficient of concordance (KCC) using RESTplus V1.25 software. To minimize individual differences, the ReHo value of each voxel was normalized by dividing it by the global mean ReHo value of the entire brain, resulting in mReHo.

Mean Amplitude of Low-Frequency Fluctuation (mALFF) and its derived metric, mean fractional ALFF (mfALFF): The time series of each voxel was transformed into the frequency domain using a Fast Fourier Transform (FFT) to obtain the power spectrum. ALFF was calculated as the square root of the power spectrum. fALFF was defined as the ratio of the ALFF value in the low-frequency range (0.01–0.08 Hz) to the ALFF value across the entire frequency range (0–0.25 Hz). To minimize individual variability, the fALFF value of each voxel was normalized by dividing it by the global mean fALFF value of the whole brain, resulting in the mfALFF.

Functional images were transformed into a mirrored space after preprocessing. The mean BOLD time series for each voxel was extracted. Pearson's correlation coefficients were computed for mirrored voxels from the bilateral hemispheres to produce VMHC maps. The Fisher-z transform was used to enable subsequent group-level comparisons.

Z-score normalized Functional Connectivity (zFC) values: Brain regions exhibiting significant between-group differences in functional metrics were defined as regions of interest (ROIs). Functional connectivity between each ROI and the entire brain was computed using the RESTplus software. The resulting correlation coefficients were subsequently standardized using Fisher's r-to-z transformation to obtain zFC values.

3.8.2 Clinical Data

Datasets: Intent-to-Treat (ITT) and Per-Protocol (PP) analyses were used.

Normally distributed continuous data were described as mean ± standard deviation, and inter-group comparisons for normally distributed variables used independent-samples t-tests. Non-normally distributed scale data will be described as medians and interquartile ranges, and the Mann–Whitney U non-parametric test will be used for statistical analysis. Categorical data were analyzed using the Chi-square test.

All statistical analyses were performed using SPSS or R software. Statistical significance was set at $P < 0.05$.

四、Ethics and Participant Protection

Before the study begins, the study protocol (including questionnaires and the informed consent form) will be submitted for review and approval to the Ethics Committee of Taizhou People's Hospital Affiliated to Nanjing Medical University (ethics number: LSKY 2025-175-01). Any amendments to the study protocol will be submitted to the Ethics Committee for approval and record-keeping. All participants will be fully informed about the study purpose, procedures, potential risks, and benefits, and will provide written informed consent.

五、References

[1] Zhang Y, Lin P, Wang R, et al. Insula-Medial Prefrontal Cortex Functional Connectivity Modulated by Transcutaneous Auricular Vagus Nerve Stimulation: An fMRI Study. IEEE J Biomed Health Inform. 2024;28(10):5962-5970.

[2] de Oliveira Rodrigues DM, Menezes PR, Machado Ribeiro Silotto AE, et al. Efficacy and Safety of Auricular Acupuncture for Depression: A Randomized Clinical Trial [J]. JAMA Netw Open. 2023;6(11):e2345138.

[3] Natbony LR, Zhang N. Acupuncture for Migraine: a Review of the Data and Clinical Insights [J]. Curr Pain Headache Rep. 2020;24(7):32.

[4] Uddin N, Levine DL. Battlefield Acupuncture for the Treatment of Chronic Migraines [J]. Cureus. 2024;16(5):e60369.

[5] Wu S, Liang J, Zhu X, et al. Comparing the treatment effectiveness of body acupuncture and auricular acupuncture in preoperative anxiety treatment[J]. J Res Med Sci. 2011;16(1):39-42.

[6] Moss DA, Crawford P. Ear Acupuncture for Acute Sore Throat: A Randomized Controlled Trial[J]. J Am Board Fam Med. 2015;28(6):697-705.

[7] Ferreira LA, Grossmann E, Januzzi E, et al. Ear Acupuncture Therapy for Masticatory Myofascial and Temporomandibular Pain: A Controlled Clinical Trial[J]. Evid Based Complement Alternat Med. 2015;2015:342507.

[8] Zhang S, He JK, Meng H, et al. Effects of transcutaneous auricular vagus nerve stimulation on brain functional connectivity of medial prefrontal cortex in patients with primary insomnia. Anat Rec (Hoboken). 2021;304(11):2426-2435.

[9] Huang Y, Zhang Y, Hodges S, et al. The modulation effects of repeated transcutaneous auricular vagus nerve stimulation on the functional connectivity of key brainstem regions along the vagus nerve pathway in migraine patients. Front Mol Neurosci. 2023;16:1160006.

[10] Qi M, Huang Y, Mai R, et al. Baseline functional connectivity of the basal forebrain-cortical circuit predict taVNS treatment response in primary insomnia: a randomized controlled trial and fMRI study. BMC Med. 2025;23(1):412.

[11] Desmond JE, Glover GH. Estimating sample size in functional MRI (fMRI) neuroimaging studies:

statistical power analyses. J Neurosci Methods. 2002 Aug 30;118(2):115–28.