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Transcutaneous vagus nerve stimulation for patients with remitted recurrent major depressive disorder: Protocol for a longitudinal neuroimaging study

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Title Page

Title: Transcutaneous vagus nerve stimulation for patients with remitted recurrent major depressive disorder: Protocol for a longitudinal neuroimaging study

Running title: taVNS therapy in preventing MDD relapse

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Site of the study

The trial site will be the Beijing Hospital of Traditional Chinese Medicine, Guang'anmen Hospital and Anding Hospital, Capital Medical University, Beijing, China.

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Author Contributions

All authors conceived and designed the study, helped write the article, and reviewed and approved it for submission.

Conflict of Interest Statement

The authors declare that they have no conflict of interests.

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Strengths and limitations of this study

- This will be the first multi-center, prospective parallel-group, patient-assessor-blinded, randomized controlled trial for taVNS in preventing major depressive disorder (MDD) episode. The participants will be individuals with recurrent remitted MDD.
- This study integrates six distinct levels of perspective to explore the effect and underlying mechanism of transcutaneous vagus nerve stimulation in the prevention of depression relapse, including the measurement of affective neuropsychology, cognition, pro-inflammatory cytokines, serum monoamine neurotransmitters (dopamine and serotonin), endocrinology (salivary cortisol for hypothalamic–pituitary–adrenal axis), and multimodal neuroimaging.
- The study design includes two control arms, which allows us to figure out the effects of taVNS in preventing MDD episode with a nontreatment control.
- The taVNS treatments requires high compliance of the patient as a self-administered method, which may lead to part of data unable meet the needs of this study. We overcome this by requiring patients keeping diaries.

Abstract

Background

After the first episode, patients with remitted major depressive disorder (MDD) have a

60% chance of experiencing a second episode. There are currently no accepted, effective methods to prevent the recurrence of MDD in remission. Transcutaneous vagus nerve stimulation (taVNS) is a noninvasive, safe, and economical approach based on the efficacy of VNS in improving clinical depression symptoms. This clinical trial will study the efficacy of taVNS in preventing MDD relapse and investigate the underlying mechanisms of this.

Methods and design

We will conduct a multicenter, randomized, patient- and evaluators double-blinded trial. We will randomize 90 eligible participants with recurrent MDD in remission in a 1:1 ratio into a real or sham taVNS group. All participants will be given six biopsychosocial assessments: pro-inflammatory cytokines, serum monoamine neurotransmitters, cognition, affective neuropsychology, multimodal neuroimaging, and endocrinology. After the baseline measurements, all participants will be given corresponding interference for 6 months and then complete a 1-year follow up. The assessments will be performed three times: at baseline, post-treatment, and at the end of 1-year follow up (except for multimodal MRI scanning, which will be conducted at the first two assessments only). Change in 17-item HAMD scores for MDD is the primary outcome parameter.

Ethics and dissemination

The study protocol was approved by the Medical Ethical Committee of Beijing Hospital

of Traditional Chinese Medicine on January 18, 2019 (2018BL-076). The trial results will be published in peer-reviewed journals and at conferences.

Trial registration number

Chinese Clinical Trial Registry (www.chictr.org.cn, ChiCTR1900022618); Pre-results.

Keywords: hypothalamic-pituitary-adrenal axis; major depressive disorder; multimodal magnetic resonance imaging; neurotransmitters; pro-inflammatory cytokines; transcutaneous vagus nerve stimulation

Introduction

Rationale

Major depressive disorder (MDD) is a chronic, costly, highly prevalent, recurrent, and debilitating psychiatric disorder characterized by low mood, loss of interest, rumination, low self-esteem, feelings of hopelessness, and even risk of suicide.[1] The Global Burden of Diseases, Injuries, and Risk Factors Study 2016 indicates that MDD causes approximately 34 million people live with disability[2] and the World Health Organization projects that the disease will rank first cause of burden by the year 2030.[3] Next to suicide and cardiovascular comorbidity, an important factor contributing to the burden of MDD is its tendency to recur in clinical management.[4] Notably, almost 50% of patients experience new depressive episodes within 2 years of recovery.[5] Even worse, the proportion of recurrences gradually increases with the increased number of recurrences; for example, 60% of MDD patients experience a first recurrence, 70% a second, and even as high as 90% a third.[6] However, the number of previous depressive episodes and cognitive- or affective-related residual symptoms, e.g., persistent subclinical depressive symptoms, rumination, impaired attentional control, and cognitive decline, have been identified as the most important clinical markers in predicting the risk factors for relapse.[7-11] Despite recent advances in pharmacological antidepressant therapy, MDD remains an incapacitating psychiatric condition with increasing prevalence and societal and economic burden.[12] Therefore, alternative treatments for full recovery from MDD are greatly needed in the field.[13]

Currently, antidepressants and cognitive behavioral therapy are still widely used treatments for MDD in clinical practice.[14,15] However, only at most 35% of MDD patients achieve remission, and the choice of antidepressants is often based on trial and error rather than identified neural pathologies.[16] Worse still, achieving remission is only the first step, and too often initially successful treatment is followed by relapse.[17] In view of such facts, vagus nerve stimulation (VNS) was approved by the U.S. Food and Drug Administration as an adjunctive long-term treatment for chronic recurrent MDD in those aged 18 years of age or older.[18] Noninvasive transcutaneous auricular VNS (taVNS) is conceptually similar to the mechanisms of VNS.[19] taVNS achieves its effects via surface skin electrodes applied in the auricular branch of the vagus nerve, known as the vagally innervated external ear regions.[20] From a neuroanatomic view, the auricular branch of the vagus nerve (ABVN) is the only branch of the vagus nerve on the body surface.[21] It projects to the nucleus tractus solitarius, which is further connected to other brain regions, such as the locus coeruleus, parabrachial nucleus, hypothalamus, thalamus, amygdala, hippocampus, anterior cingulate cortex, anterior insula, and lateral prefrontal cortex.[22]

A systematic review written by Redgrave et al. showed that the side effects of taVNS were local skin irritation, headache, nasopharyngitis, and some possible serious adverse events (e.g., palpitations).[23] Considering that the ABVN projects to the parabrachial nucleus, which can regulate heart rate, some studies showed taVNS could have side effects on heart rate at specific parameters (pulse width 500μs and frequency 25Hz) [24].

For most cases, the side effect was not obvious or just mild and disappeared after follow up. [25-27]

Given the importance of taVNS in producing a beneficial antidepressant response, neuroimaging studies in patients with mild to moderate MDD have demonstrated that taVNS alters functional connectivity in the default mode network.[28,29] Furthermore, insula activation is correlated with the clinical effectiveness of taVNS treatment.[30] Likewise, hypoconnectivity between the bilateral medial hypothalamus and rostral anterior cingulate cortex (rACC) as well as hyperconnectivity between the left nucleus accumbens and bilateral rACC during 4 weeks of taVNS treatment have been reported.[31,32] Taken together, these studies indicate that taVNS has the potential to treat mild to moderate MDD and modulate a wide range of resting-state nodes distributed throughout a wide range of neural networks, including the default mode network, salience network (insula), and the reward network.[29]

Our previous study demonstrated that chronic inflammation and dysregulation of the immune system are inherent characteristics of recurrent MDD.[6] The conditions associated with chronic inflammation and stress can induce activation of the hypothalamic-pituitary-adrenal (HPA) axis, impair the functions of neurotransmitters, alter brain circuits, and contribute to the recurrence of MDD.[33,34] Studies have shown that hyperactivity of the HPA axis often results in hypercortisolism, which is associated with increased vulnerability to MDD relapse.[35] It is also important to note that some pro-inflammatory cytokines, such as interleukins (e.g., IL-1, IL-2, and IL-6) and tumor

necrosis factor-α (TNF-α) can lead to depressive behavioral symptoms and changes in the course of MDD through various pathways.[36] Pro-inflammatory cytokines can reduce the level of 5-hydroxytryptamine (5-HT or serotonin) by affecting tryptophan metabolism and increase neurotoxic metabolites (such as 3-hydroxyguanosine and quinolinic acid) through promotion of the kynurenine pathway.[37,38] Moreover, the decrease of monoamine neurotransmitters, such as 5-HT, dopamine (DA), and norepinephrine (NE), are risk factors for the etiology and pathophysiological mechanisms of MDD.[39] As a result, it is suggested that taVNS may affect the HPA axis, pro-inflammatory cytokines, neurotransmitters, and brain circuits and thus prevent MDD relapse.

Since taVNS has been shown to be effective in the treatment of mild to moderate MDD, in this study we aimed to prospectively prevent remitted MDD relapse using taVNS and explore the underlying mechanisms of this.

Study aims and theoretical framework

Based on the above, we integrate aspects of theories from six distinct perspectives to explore the effects and underlying mechanisms of taVNS in preventing depression relapse: pro-inflammatory cytokines, serum monoamine neurotransmitters, cognition, affective neuropsychology, multimodal neuroimaging, and endocrinology (HPA axis and monoamine neurotransmitters) (Figure 1).

The present study aims to 1) determine the efficacy of taVNS in preventing MDD

recurrence; 2) elucidate the neural mechanisms of taVNS; and 3) explore the relationships between the HPA axis, pro-inflammatory cytokines, neurotransmitters, and brain circuits.

Hypotheses

1. We hypothesize that the recurrence rate of remitted MDD will be significantly improved in the taVNS treatment group versus the sham group, as assessed by 17-item Hamilton Depression Rating Scale (HAM-D) scores for MDD during 6-month treatment and at 1-year follow up.

2. We hypothesize that taVNS can significantly alter HPA-axis activity, reduce inflammation, increase levels of monoamine neurotransmitters (e.g., 5-HT, DA), and change gray/white matter structure and function compared with sham taVNS.

Methods

Design

The trial site will be the Beijing Hospital of Traditional Chinese Medicine, Guang'anmen Hospital, and Beijing Anding Hospital. The present study will be conducted as a multicenter, prospective parallel-group, patient-assessor-blinded, randomized controlled trial, consisting of two stages. First, we will obtain baseline measures, including demographic information, neuropsychological scales, multimodal MRI scans, HPA axis markers, pro-inflammatory cytokines, and monoamine neurotransmitters. Second, ninety remitted recurrent MDD patients will be randomly

assigned to 6-month treatment of taVNS or sham taVNS in a 1:1 ratio. At the end of treatment, all participants will be required to complete above baseline measurements and we will examine whether 6 months of taVNS can significantly reduce inflammation, alter HPA axis activity, increase neurotransmitters, and regulate brain circuits compared with sham taVNS. Third, those participants who complete the second measurements be followed up clinically for 1 year. Finally, at the end of the follow up, we will invite the respective participants to repeat the baseline measures again except for the multimodal MRI scans (Figure 2). Of note, the clinical scales and bioassays will be obtained in the hospital where the participants were recruited, the multimodal MRI data will be obtained at Beijing Normal University. This study protocol is presented according to the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) guidelines.[40]

Sample size estimation

Previous studies have shown that the effective rate of taVNS in the treatment of MDD is about 80% and that of the false taVNS group is about 45%.[41,42] Based on an alpha error of 0.05 and beta of 0.1, a minimum sample size of 35 patients was calculated for each group. With an estimated about 20% drop out during follow up, we will recruit about 45 participants to each group.

Inclusion criteria

All patients will meet the following criteria: (1) ages between 18 and 60 years; (2) right-

handed; (3) history of remitted recurrent MDD, implying more than two previous depressive episodes as assessed using the DSM-IV structured clinical interview and are in a remitted state (≥8 weeks assessed by the 17-item HAM-D ≤ 7);[4] (4) no history of neurologic or other chronic medical diseases; (5) no history of other psychiatric disorders such as schizophrenia or obsessive-compulsive disorder; (6) no history of stimulant use for MDD; and (7) no history of alcohol or substance abuse.

Exclusion criteria

The exclusion criteria will be as follows: 1) ongoing addiction to drugs and alcohol; 2) previous head injury; 3) a family history of psychiatric illness; 4) obvious mental retardation (Mini-Mental State Examination ≥ 27) or dementia; 5) current pregnancy or breastfeeding; 6) any contraindications to an MRI scan; 7) failure to agree to signing the consent form.

Patient and public involvement

Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Ethics

We will follow according to the Declaration of Helsinki principles (Seoul, October 2008) to conduct this study. The study protocol was approved by the Medical Ethical Committee of Beijing Hospital of Traditional Chinese Medicine on December 3, 2018 (2018BL-076). Written informed consent will be obtained from each participant. If

desired, we will give participants up to 2 weeks to consider their decision. All investigators will receive good clinical practice training. We will anonymize and encrypt the raw data. Only researchers directly involved in the study will have access to data.

Measures

First, demographic information will be compiled for study participants, such as gender, age, marital status, education, contact information, etc. Second, the relevant epidemiological data will also be collected, including smoking, drinking, substance abuse, family history of mental illness, age of first onset, duration of the first episode, number of previous depressive episodes, illness duration, changes in appetite, rhythm of life, and dosage and duration of medication.

Cognitive assessments

We will evaluate neurocognitive function, including memory, attention, processing speed, and executive function, using the Cambridge Neuropsychological Test Automatic Battery, Trail Making Test, and Wisconsin Card Sorting Test.[43]

Affective neuropsychological assessments

The 17-item HAM-D will be used to assess the severity of patient depression and the HAM-A to measure anxiety.[44] The Rumination Response Scale will be used to measure the severity of rumination symptoms and the Dysfunctional Attitude Scale will be used to measure the intensity of dysfunctional attitudes.[45] Each questionnaire will be

completed on the day of scanning, at the end of taVNS treatment, and at the last day of 1-year follow up, respectively.

Blood measures

Pro-inflammatory cytokines

Fasting peripheral venous blood samples (5 mL) will be collected in tubes treated with ethylenediaminetetraacetic acid (EDTA) (S-Monovette, Sarstedt, Nümbrecht, Germany) at 08:00 by venipuncture. Plasma will be immediately separated by centrifugation (2000 *g*, 10 min, 4℃) and stored at -80℃ until analysis. IL-1, IL-6, IL-8 and TNF-α concentrations will be measured by the enzyme-linked immunosorbent assay (ELISA) (Human Quantikine ELISA, R&D Systems, Minneapolis, MN, USA) according to the manufacturer's protocols. Assay sensitivity will be 0.70 pg/ml.

Monoamine neurotransmitters

Blood samples of all participants will be collected at the three time-points (baseline, after treatment, and end of follow up). We will draw 10 mL of antecubital vein blood from each participant from the left arm and collect it in vacutainer tubes containing 0.5 mM EDTA. The whole blood samples will be fractionated by centrifugation at 2000 r/min for 10 min at room temperature as soon as they are delivered to the laboratory. After centrifugation, serum will be separated into the upper layer and then individually transferred to a clean tube. All serum samples will be immediately stored at −80°C until analysis. The dopamine concentration will be determined using the high-performance liquid chromatographic (HPLC) method with the Acclaim HPLC (Bio-Rad, USA).[46]

Serotonin concentration will be determined by HPLC with electrochemical detection, utilizing an internal standard (N-methyl-5HT).[47]

Salivary cortisol for HPA axis markers

Participants will also provide a saliva sample to assess the activity of the HPA axis before the MRI scan. Eating, drinking, smoking, or brushing teeth in the previous 15 min will not be allowed. We will instruct participants to provide five saliva samples over a day (at awakening, 30, 45, and 60 min thereafter, followed by a fifth measurement at 22:00 h) to reflect the diurnal morning awakening curve and evening HPA-axis activity. Salivette® (Sarstedt AG & Co., Nümbrecht, Germany) containers will be used to contain the saliva samples. After receipt, Salivette containers will be stored at -20ºC and later sent to centrifugation (3000 rpm for 5 min) and aliquotation, after which they will be frozen at -20ºC until analysis by ELISA (IBL International, Hamburg, Germany) to determine salivary cortisol levels.[48]

Change in 17-item HAMD scores for MDD is the primary outcome parameter, change in cognition scales and bioassays are the secondary outcome parameters.

Multimodal MRI scanning procedure

Acquisition of MRI brain imaging data will be conducted using a Siemens Skyra 3-Tesla scanner (Siemens, Erlangen, Germany) in Guang'anmen Hospital, China Academy of Chinese Medical Sciences, Beijing. T1-weighted sagittal high-resolution structural images will be acquired with the three-dimensional fast spoiled gradient-echo

sequence: repetition time/echo time (TR/TE), 2,530 ms/3.39 ms; thickness/gap, 1.0/0 mm; matrix, 256 × 256; voxel size, 1 × 1 × 1 mm^3; and 9° flip angle (FA). Resting-state functional MRI (rs-fMRI) encompassing the whole brain will be obtained using an echo-planar imaging sequence: TR/TE, 2000 ms/30 ms; 90° flip angle; matrix, 64 × 64; thickness/gap, 4.0 mm/0.6 mm, field of view (FOV), 232 × 232 mm^2; 8 min; paralleled by anterior commissure-posterior commissure line. All participants will receive the following instructions: stay awake, do not move, close your eyes, and do not think about anything. Diffusion tensor images (DTI) will be acquired via an echo-planar imaging sequence using the following parameters: TR/TE = 11000 ms/98 ms, FOV = 256 × 256 mm^2, matrix = 128 × 128, slice thickness = 2 mm and 60 continuous axial slices without a gap. The diffusion sensitizing gradients will be applied to 12 nonlinear directions (b = 1,000 s/mm^2), together with an acquisition image without diffusion weighting (b = 0 s/mm^2). For anatomic reference, T1 images will be obtained before resting-state scans.

Blinding and randomization

After completion of baseline assessments, group assignment will be determined by opening an opaque envelope (through a research assistant), revealing the participant's randomised assignment to one of the two groups. Randomization is based on random numbers generated from random number tables. These researchers will be independent from the members of the study who are responsible for enrolling the participants. Patients in the real and sham taVNS condition will be blinded (until they finished the study), as well as the assessment of taVNS effectiveness. The group assignment list will

be withheld until the final evaluation of the study data. All measurements will be performed by blinded experimenters.

Interventions

After MRI scanning, all participants will be trained to apply taVNS or sham taVNS. All subsequent interventions will be self-administered by the patients at home.[49] The taVNS treatment will be terminated when participants experienced intolerable symptoms, e.g., pain at stimulus points, dizziness, etc.

Real taVNS group

Location: The points for taVNS are in the auricular concha area where there are rich vagus nerve branch distributions. taVNS will be applied to the concha area of both ears simultaneously during treatment.

Intervention procedure: Patients will take a seated position or lie on their sides. After the stimulation points are disinfected according to standard practice, ear clips will be attached to the ear area (auricular concha) at the stimulation site. Stimulation parameters will include: 1) density wave adjusted to 20 Hz with a wave width less than 1 ms and 2) intensity adjusted based on the tolerance of the patient (4–6 mA). Each stimulation will last for 30 min and be completed twice a day (once in the morning and once again in the evening). The treatment will last for 5 days each week with 2 days

off.

Sham taVNS group

Location: The stimulation points for sham taVNS are located at the superior scapha (outer ear margin midpoint) where there is no vagus nerve distribution. Similar to taVNS, sham taVNS will be applied on both ears simultaneously during the treatment.

Follow-up procedure

We will follow up the remitted recurrent MDD participants every 3 months and repeat the baseline measurements (except the multimodal MRI scan). To maximize recurrence detection rates, we will also instruct participants to contact us if recurrence occurs.

Data management

All the affective neuropsychology, cognition, pro-inflammatory cytokines, serum monoamine neurotransmitters, endocrinology, and multimodal neuroimaging data will be anonymized and upload on dedicated servers (http://www.bjzhongyi.com/) within six months after the trail complete.

MRI data preprocessing

The T1 data will be processed using Freesurfer (http://surfer.nmr.mgh.harvard.edu). The processing procedures include motion correction, skull stripping, segmentation of white matter, creation of the pial surface and surface of the white/gray junction, inflation of the folding surface plane, and topology correction. All the above procedures

will be completed automatically. Parameters such as global cortical structure, whole-brain cortical thickness, volume and surface area, and so on, will be obtained through this procedure.[50]

Resting-state functional MRI (rs-fMRI) data will be preprocessed using Data Processing and Analysis for Brain Imaging (DPABI, http://www.rfmri.org/dpabi) based on Statistical Parametric Mapping (SPM12, http://www.fil.ion.ucl.ac.uk/spm). The rs-fMRI data will undergo slice-timing correction, motion correction, scaling to percent signal change, smoothing with a Gaussian kernel of 6 mm full-width-at-half maximum, bandpass temporal filtering (0.01–0.1 Hz), and grand mean intensity normalized. We will evaluate the brain circuits through regional homogeneity (ReHo), amplitude of low-frequency fluctuations (ALFF), fractional ALFF (fALFF), functional connectivity, independent component analysis, and graph theoretical network analyses, and so on.

DTI data will be preprocessed using the Pipeline for Analyzing braiN Diffusion imAges (PANDA, https://www.nitrc.org/projects/panda/) based on Functional MRI of the Brain (FMRIB's) Software Library (FSL) tools (http://www.fmrib.ox.ac.uk/fsl). The preprocessing procedures include converting DICOM files into NIFTI images, estimating the brain mask, cropping the raw images, correcting for the eddy-current effect, averaging multiple acquisitions, calculating diffusion tensor (DT) metrics. The DT metrics include fractional anisotropy (FA), mean diffusivity, axial diffusivity, and radial diffusivity. The four DTI-metric images were then normalized to the MNI space

with a 1-mm spatial resolution template and outputted for further analysis. Several voxel-based analyses will be applied for the above four parameters to reflect the change of white matter microstructure. In addition, we will also conduct Tract-Based Spatial Statistics (TBSS) analysis to avoid false results caused by spatial smoothing. For TBSS analysis, all the aligned FA images will be skeletonized and a mean FA skeleton generated, then the individual images with data on the skeleton will be created for voxel-wise statistical analysis on the skeleton. Statistical analyses will be performed using nonparametric permutation testing (Randomize in FSL) with 5000 Monte Carlo simulations. We will evaluate the structural differences in each parameter above between the groups accounting for age and head motion.[51]

Distributions and missing data

We will inspect distributions and remove outliers and data noncompliant to the protocol (e.g., saliva samples that significantly exceed the time limit). We will transform non-normally distributed data where possible, otherwise we will apply non-parametric tests or bootstrapping if applicable. For extensive missing data at random, we will use multiple imputation where necessary and possible. For those missing data that affect the analysis, we will discard it.

Statistical analysis plan

Statistical analysis will be performed using SPSS 22 Software (SPSS Inc., Chicago, IL, USA). The statistical tests will be two-sided with 5% significance level. Means and

standard deviations will be used for the statistical description of continuous variables. For group analysis of each bioassay, Shapiro-Wilkes tests of distribution normality will first be performed, and those with non-Gaussian distributions will be either log-transformed or analyzed using non-parametric tests. A two-sample t-test and a chi-square test will be applied to compare the baseline characteristics of the participants between groups. For longitudinal data, different statistical methods were applied for different purpose. For hypothesis one, the paired *t* test will be applied to detect the difference of 17-item HAM-D scores of each group for baseline *vs.* treatment, as well as other index. For hypothesis two, the analysis of covariance (ANCOVA) will be used for the comparison of each period and each group. Bonferroni correction ($P < 0.05/3$) will be performed to compare every two different periods as a post hoc test if the variance analysis test result is significant. Finally, we will compute correlations between these neural function imaging indicators, cognitive scores, neuropsychological scores, levels of pro-inflammatory cytokines, monoamine neurotransmitters, and salivary cortisol.

Benefits and risk assessment

We focused on depressed patients in remission, who do not require antidepressant treatment measures but at risk of episode. Some of the participants will benefit more or less from this study to reduce potential recurrence. In addition, the advantage of follow-up is that the recurrence of MDD can be detected timely, so as to provide timely psychiatric treatment. In case of an emergency occurred during follow-up, such as

suicide, we have an addition protocol available including a consulting psychiatrist for emergency situations and referral the most appropriate emergency service.

Compensation

In addition to travel cost compensation, participants will receive ¥ 200. For completion of a follow-up scan we will pay ¥ 100,

Discussion

Summary

In summary, the current study will investigate the efficiency of taVNS in preventing MDD relapse and its mechanisms, focusing on multidimensions, e.g., brain circuits, inflammation status, monoamine neurotransmitters, and endocrine (glucocorticoids for HPA axis status), by comparing real versus sham taVNS intervention. We also examine the relationship between the change in depressive symptoms and the above multidimensional parameters, to determine predictive biomarker(s). The cohort of recurrent MDD participants will be followed up to test to what extent baseline measurements are predictive and/or how they change prospectively before recurrence. This will help elucidate the neural mechanisms underlying taVNS prevention of MDD relapse and open up the possibility of targeting novel therapeutic strategies that provide a safe and effective method with fewer side effects for the prevention of MDD relapse.

Limitations

This present study has several limitations. First, taVNS is a self-administered treatment

whereby patient compliance may influence clinical outcome. To enhance compliance, we will require all patients to complete daily diary entries, including details of side effects and improvement. In addition, we will check all the diaries through regular assessments and offer both telephone and face-to-face advisory sessions during the entire treatment period. Despite this potential limitation, this kind of self-administered therapy provides a good choice for patients because of its feasibility and efficacy, and it also significantly reduces treatment expenses and time costs. Second, to overcome the potential confounding effects of antidepressants and other psychotropic medication, only participants who currently do not use these drugs will be included. Of note, we will not include single-episode MDD participants.

Conclusion

By integrating assessments of pro-inflammatory cytokines, cognition, affective neuropsychology, multimodal neuroimaging, and endocrinology (specifically the HPA axis and monoamine neurotransmitters) using a prospective repeated-measures design in remitted recurrent MDD participants, the present study will provide further insight into the mechanisms of taVNS in preventing MDD relapse. Increased insight will lead to the identification of novel targets for (1) improved preventive therapy, and/or (2) (bio)markers to monitor and/or predict recurrence risk. Ultimately, this study methodology holds the potential to alleviate MDD's highly recurrent course and reduce its currently overwhelming global disease burden.

Trial status

The Medical Ethical Committee of the Beijing TCM Hospital approved the study protocol on January 18, 2019 (authorization 2018BL-076). This trial has been registered since April 19, 2019 (Registration Number ChiCTR1900022618). The trial started on May 11, 2019. The first participant was studied on May 15, 2019, and 60 participants have been recruited as of the date of this submission. The trial is currently recruiting participants. We predict that recruitment will be completed by October 2021.

Author Contributions

All authors conceived and designed the study, helped write the article, and reviewed and approved it for submission.

Figure legends

Figure 1. Theoretical framework. The blue part of Figure 1 illustrates the main theories of major depressive disorder (MDD) related to the hypothalamic-pituitary-adrenal (HPA) axis, pro-inflammatory cytokines, and monoamine neurotransmitters. The red part of Figure 1 shows the possible mechanism of taVNS in preventing MDD recurrence. It is proposed that stress, chronic inflammation, and weakening of the immune system induce activation of the HPA axis, and that impaired negative feedback induces the sustained rise of glucocorticoids and its resistant, then promotes the elevation of pro-inflammatory cytokines. Furthermore, it is proposed that some peripheral pro-inflammatory cytokines can act on neurons and supporting cells to not

only cross the blood-brain barrier directly (e.g., astrocytes and microglia), but also elicit depressive-like behaviors through afferent signals pathways (e.g., glucocorticoid receptors and monoamine neurotransmitters). taVNS may inhibit MDD episodes by affecting brain circuits and reducing inflammation through effects on pro-inflammatory cytokines.

Figure 2. Study design. Figure 2 depicts the design of the present study. Different parts of the study are shown in chronological order from left to right. Recruited patients and controls will participate in the initial assessment for inclusion and exclusion criteria, relevant demographic information, and other variables. After the baseline data are collected, we will take blood and saliva samples and conduct neuropsychological tests. Subsequently, participants will have a magnetic resonance imaging (MRI) session where we will perform structural (T1-weighted and diffuse tensor imaging) and resting-state functional MRI scans. Then, we will separate participants randomly into the real and sham transcutaneous auricular vagus nerve stimulation groups. After the 6-month intervention, we will monitor the patients by calling them every ~4 months to assess recurrence. In cases where we detect a recurrence, we will invite the respective patient—together with matched patients without recurrence—to repeat some of the baseline assessments (blood samples, neuropsychological tests, and multimodal MRI).

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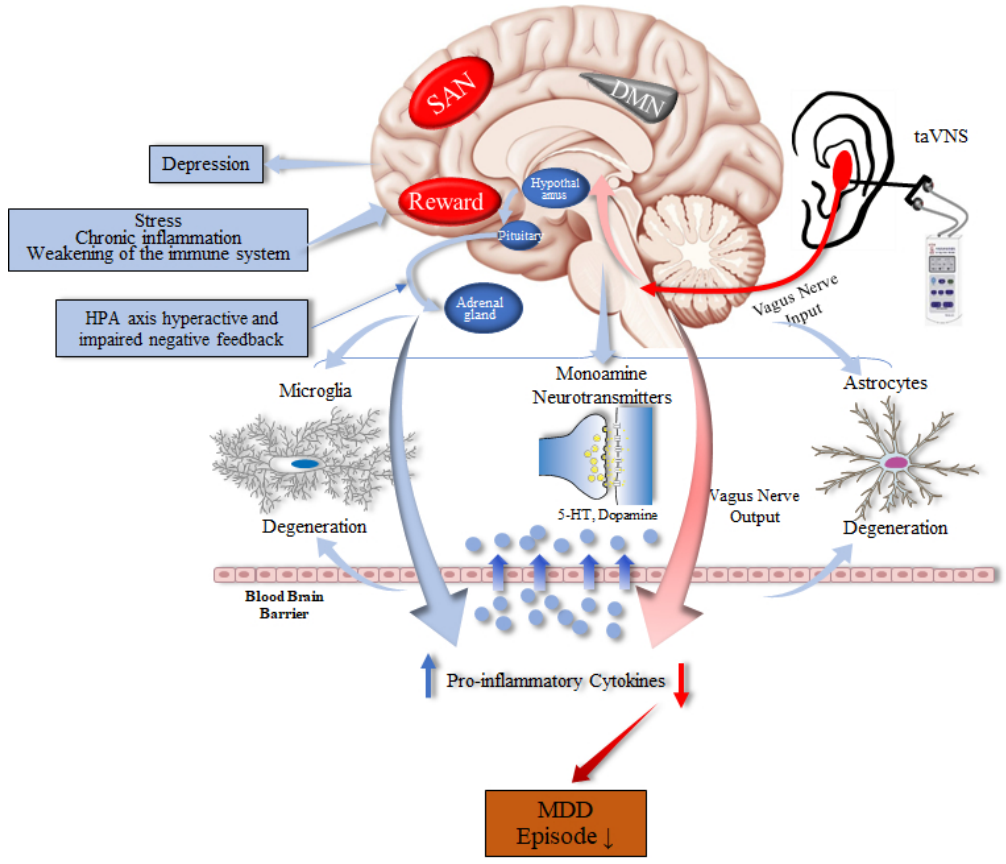

Figure 1 Theoretical framework. The blue part of Figure 1 illustrates the main theories of major depressive disorder (MDD) related to the hypothalamic-pituitary-adrenal (HPA) axis, pro-inflammatory cytokines, and monoamine neurotransmitters. The red part of Figure 1 shows the possible mechanism of taVNS in preventing MDD recurrence. It is proposed that stress, chronic inflammation, and weakening of the immune system induce activation of the HPA axis, and that impaired negative feedback induces the sustained rise of glucocorticoids and its resistant, then promotes the elevation of pro-inflammatory cytokines. Furthermore, it is proposed that some peripheral pro-inflammatory cytokines can act on neurons and supporting cells to not only cross the blood-brain barrier directly (e.g., astrocytes and microglia), but also elicit depressive-like behaviors through afferent signals pathways (e.g., glucocorticoid receptors and monoamine neurotransmitters). taVNS may inhibit MDD episodes by affecting brain circuits and reducing inflammation through effects on pro-inflammatory cytokines.

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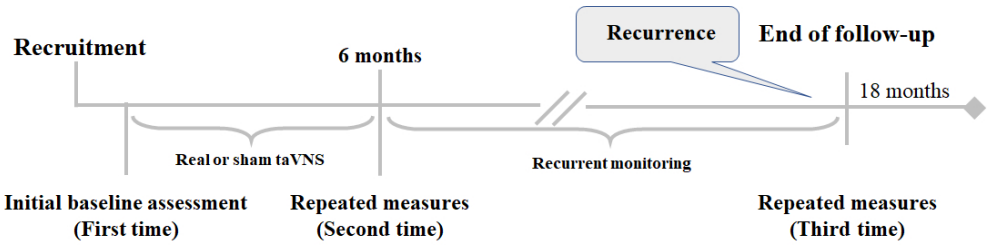

Figure 2. Study design. Figure 2 depicts the design of the present study. Different parts of the study are shown in chronological order from left to right. Recruited patients and controls will participate in the initial assessment for inclusion and exclusion criteria, relevant demographic information, and other variables. After the baseline data are collected, we will take blood and saliva samples and conduct neuropsychological tests. Subsequently, participants will have a magnetic resonance imaging (MRI) session where we will perform structural (T1-weighted and diffuse tensor imaging) and resting-state functional MRI scans. Then, we will separate participants randomly into the real and sham transcutaneous auricular vagus nerve stimulation groups. After the 6-month intervention, we will monitor the patients by calling them every ~4 months to assess recurrence. In cases where we detect a recurrence, we will invite the respective patient—together with matched patients without recurrence—to repeat some of the baseline assessments (blood samples, neuropsychological tests, and multimodal MRI).

93x23mm (300 x 300 DPI)

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

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In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

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		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet	3,23

		registered, name of intended registry	
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	N/A It's not a World Health Organization Trial Registration Data Set.
Protocol version	#3	Date and version identifier	23
Funding	#4	Sources and types of financial, material, and other support	2
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1,2
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	1
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	N/A The sponsor and funder are not involved in this study.
Roles and	#5d	Composition, roles, and responsibilities of the	N/A

responsibilities: committees		coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	7-11
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	11
Objectives	#7	Specific objectives or hypotheses	11
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	11-12
Methods: Participants, interventions, and outcomes			

Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	11-12
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	12-13
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	16-17
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	14
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	4,19
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	N/A There were no relevant

			concomitant care and interventions that are permitted or prohibited during the trial.
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	2,16
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	11, and 23 Figure 2
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	12
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	12

Methods:			
Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	17
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	17
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	17
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	17

Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	17
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	13
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	22
Data management	#19	Plans for data entry, coding, security, and	13

		storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	18-19
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	N/A Additional statistical analyses will be conducted on a case-by-case basis after trial data collection is complete.
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	21
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting	N/A

		structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	The study will not have a formal DMC since adverse intervention events have not been reported.
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	18
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	N/A
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	N/A
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	N/A Because it has been approved by the ethic

			committee of Beijing Hospital of Traditional Chinese Medicine.
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	N/A There is no above corresponding plan by now.
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	13
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	13
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	N/A There were on conflict interests to others.

Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	13
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	22
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	6
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	N/A
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	19
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	Please refer to the supplementary Patient Consent Form

Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	N/A

BMJ Open

The effect and neural mechanisms of the transcutaneous vagus nerve stimulation for relapse prevention in patients with remitted major depressive disorder: Protocol for a longitudinal study

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Title Page

Title: The effect and neural mechanisms of the transcutaneous vagus nerve stimulation for relapse prevention in patients with remitted major depressive disorder: Protocol for a longitudinal study

Running title: taVNS therapy in preventing MDD relapse

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Site of the study

The trial site will be the Beijing Hospital of Traditional Chinese Medicine, Guang'anmen Hospital and Anding Hospital, Capital Medical University, Beijing, China.

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Author Contributions

CHL and LW contributed to the conception of the study. The protocol was drafted by ZQZ and was reviewed by LW and CHL. ZQZ, ZPG and XXW will supervise the trial. XXW was applied for the ethical approval. PS provided the guidance of statistical analysis. ZQZ and ZPG designed and drew the figures. All authors reviewed and approved the publication of the protocol.

Conflict of Interest Statement

The authors declare that they have no conflict of interests.

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The effect and neural mechanisms of the transcutaneous vagus nerve stimulation for relapse prevention in patients with remitted major depressive disorder: Protocol for a longitudinal study

Abstract

Introduction

After the first episode, patients with remitted major depressive disorder (MDD) have a 60% chance of experiencing a second episode. There are currently no accepted, effective methods to prevent the recurrence of MDD in remission. Transcutaneous vagus nerve stimulation (taVNS) is a noninvasive, safe, and economical approach based on the efficacy of VNS in improving clinical depression symptoms. This clinical trial will study the efficacy of taVNS in preventing MDD relapse and investigate the underlying mechanisms of this.

Methods and analysis

We will conduct a multicenter, randomized, patient- and evaluators double-blinded trial. We will randomize 90 eligible participants with recurrent MDD in remission in a 1:1 ratio into a real or sham taVNS group. All participants will be given six biopsychosocial assessments: pro-inflammatory cytokines, serum monoamine neurotransmitters, cognition, affective neuropsychology, multimodal neuroimaging, and endocrinology. After the baseline measurements, all participants will be given corresponding interference for 6 months and then complete a 1-year follow up. The assessments will be performed three times: at baseline, post-treatment, and at the end of 1-year follow up (except for multimodal MRI scanning, which will be conducted at the first two assessments only). Change in 17-item HAM-D scores for MDD is the primary outcome parameter.

Ethics and dissemination

The study protocol was approved by the Medical Ethical Committee of Beijing Hospital of Traditional Chinese Medicine on January 18, 2019 (2018BL-076). The trial is registered at the Chinese Clinical Trial Registry (www.chictr.org.cn,

ChiCTR1900022618). The trial results will be published in peer-reviewed journals and at conferences.

Trial registration number

Chinese Clinical Trial Registry (www.chictr.org.cn, ChiCTR1900022618); Pre-results.

Keywords: hypothalamic-pituitary-adrenal axis; major depressive disorder; multimodal magnetic resonance imaging; neurotransmitters; pro-inflammatory cytokines; transcutaneous vagus nerve stimulation

Strengths and limitations of this study

- This will be the first prospective, doubled-blinded, randomized controlled trial on taVNS to prevent the relapse of major depressive disorder (MDD).
- This study integrates six distinct types of measures from clinical symptoms, neuropsychological battery, inflammation, HPA axis activity, peripheral neurotransmitter levels to neuroimaging to examine the effects and underlying mechanism of taVNS in preventing MDD relapse.
- The study design included two control arms, which had better contrasts than using one control group.
- The self-administered taVNS treatments requires high compliance of the patients, which may influence clinical outcomes.

Introduction

Rationale

Major depressive disorder (MDD) is a chronic, costly, highly prevalent, recurrent, and debilitating psychiatric disorder characterized by low mood, loss of interest, rumination, low self-esteem, feelings of hopelessness, and even risk of suicide. [1] The Global Burden of Diseases, Injuries, and Risk Factors Study 2016 indicates that MDD causes approximately 34 million people live with disability [2] and the World Health Organization projects that the disease will rank first cause of burden by the year 2030. [3] Next to suicide and cardiovascular comorbidity, an important factor contributing to the burden of MDD is its tendency to recur in clinical management. [4] Notably, almost 50% of patients experience new depressive episodes within 2 years of recovery.[5] Even worse, the proportion of recurrences gradually increases with the increased number of recurrences; for example, 60% of MDD patients experience a first recurrence, 70% a second, and even as high as 90% a third. [6] However, the number of previous depressive episodes and cognitive- or affective-related residual symptoms, e.g., persistent subclinical depressive symptoms, rumination, impaired attentional control, and cognitive decline, have been identified as the most important clinical markers in predicting the risk factors for relapse. [7-11] Despite recent advances in pharmacological antidepressant therapy, MDD remains an incapacitating psychiatric condition with increasing prevalence and societal and economic burden. [12] Therefore, alternative treatments for full recovery from MDD are greatly needed in the field. [13]

Currently, antidepressants and cognitive behavioral therapy are still widely used treatments for MDD in clinical practice. [14 15] However, only at most 35% of MDD patients achieve remission, and the choice of antidepressants is often based on trial and error rather than identified neural pathologies. [16] Worse still, achieving remission is only the first step, and too often initially successful treatment is followed by relapse. [17] In view of such facts, vagus nerve stimulation (VNS) was approved by the U.S. Food and Drug Administration as an adjunctive long-term treatment for chronic recurrent MDD in those aged 18 years of age or older. [18] Noninvasive transcutaneous auricular

VNS (taVNS) is conceptually similar to the mechanisms of VNS. [19] taVNS achieves its effects via surface skin electrodes applied in the auricular branch of the vagus nerve, known as the vagally innervated external ear regions. [20] From a neuroanatomic view, the auricular branch of the vagus nerve (ABVN) is the only branch of the vagus nerve on the body surface. [21] It projects to the nucleus tractus solitarius, which is further connected to other brain regions, such as the locus coeruleus, parabrachial nucleus, hypothalamus, thalamus, amygdala, hippocampus, anterior cingulate cortex, anterior insula, and lateral prefrontal cortex. [22]

A systematic review written by Redgrave et al. showed that the side effects of taVNS were local skin irritation, headache, nasopharyngitis, and some possible serious adverse events (e.g., palpitations). [23] Considering that the ABVN projects to the parabrachial nucleus, which can regulate heart rate, some studies showed taVNS could have side effects on heart rate at specific parameters (pulse width 500μs and frequency 25Hz). [24] For most cases, the side effect was not obvious or just mild and disappeared after follow up. [25-27]

Given the importance of taVNS in producing a beneficial antidepressant response, neuroimaging studies in patients with mild to moderate MDD have demonstrated that taVNS alters functional connectivity in the default mode network. [28 29] Furthermore, insula activation is correlated with the clinical effectiveness of taVNS treatment. [30] Likewise, hypoconnectivity between the bilateral medial hypothalamus and rostral anterior cingulate cortex (rACC) as well as hyperconnectivity between the left nucleus accumbens and bilateral rACC during 4 weeks of taVNS treatment have been reported. [31 32] Taken together, these studies indicate that taVNS has the potential to treat mild to moderate MDD and modulate a wide range of resting-state nodes distributed throughout a wide range of neural networks, including the default mode network, salience network (insula), and the reward network. [29]

Our previous study demonstrated that chronic inflammation and dysregulation of the immune system are inherent characteristics of recurrent MDD. [6] The conditions associated with chronic inflammation and stress can induce activation of the

hypothalamic-pituitary-adrenal (HPA) axis, impair the functions of neurotransmitters, alter brain circuits, and contribute to the recurrence of MDD. [33 34] Studies have shown that hyperactivity of the HPA axis often results in hypercortisolism, which is associated with increased vulnerability to MDD relapse.[35] It is also important to note that some pro-inflammatory cytokines, such as interleukins (e.g., IL-1, IL-2, and IL-6) and tumor necrosis factor-α (TNF-α) can lead to depressive behavioral symptoms and changes in the course of MDD through various pathways.[36] Pro-inflammatory cytokines can reduce the level of 5-hydroxytryptamine (5-HT or serotonin) by affecting tryptophan metabolism and increase neurotoxic metabolites (such as 3-hydroxyguanosine and quinolinic acid) through promotion of the kynurenine pathway.[37 38] Moreover, the decrease of monoamine neurotransmitters, such as 5-HT, dopamine (DA), and norepinephrine (NE), are risk factors for the etiology and pathophysiological mechanisms of MDD. [39] As a result, it is suggested that taVNS may affect the HPA axis, pro-inflammatory cytokines, neurotransmitters, and brain circuits and thus prevent MDD relapse.

Since taVNS has been shown to be effective in the treatment of mild to moderate MDD, in this study we aimed to prospectively prevent remitted MDD relapse using taVNS and explore the underlying mechanisms of this.

Study aims and theoretical framework

Based on the above, we integrate aspects of theories from six distinct perspectives to explore the effects and underlying mechanisms of taVNS in preventing depression relapse: pro-inflammatory cytokines, serum monoamine neurotransmitters, cognition, affective neuropsychology, multimodal neuroimaging, and endocrinology (HPA axis and monoamine neurotransmitters) (Figure 1).

The present study aims to 1) determine the effects of taVNS in preventing MDD recurrence; 2) elucidate the neural mechanisms of taVNS; and 3) explore the association between the clinical outcomes and brain circuits changes.

Hypotheses

1. We hypothesize that the recurrence rate of remitted MDD will be significantly

reduced in the taVNS treatment group versus the sham group, as assessed by 17-item Hamilton Depression Rating Scale (HAM-D) scores for MDD during 6-month treatment and at 1-year follow up.

2. We hypothesize that taVNS can significantly alter HPA-axis activity, reduce inflammation, increase levels of monoamine neurotransmitters (e.g., 5-HT, DA), and change gray/white matter structure and function compared with sham taVNS.

Methods

Design

The trial site will be the Beijing Hospital of Traditional Chinese Medicine, Guang'anmen Hospital, and Beijing Anding Hospital. The present study will be conducted as a multicenter, prospective parallel-group, patient-assessor-blinded, randomized controlled trial, consisting of two stages. First, we will obtain baseline measures, including demographic information, neuropsychological scales, multimodal MRI scans, HPA axis markers, pro-inflammatory cytokines, and monoamine neurotransmitters. Second, ninety remitted recurrent MDD patients will be randomly assigned to 6-month treatment of taVNS or sham taVNS in a 1:1 ratio. At the end of treatment, all participants will be required to complete above baseline measurements and we will examine whether 6 months of taVNS can significantly reduce inflammation, alter HPA axis activity, increase neurotransmitters, and regulate brain circuits compared with sham taVNS. Third, those participants who complete the second measurements be followed up clinically for 1 year. Finally, at the end of the follow up, we will invite the respective participants to repeat the baseline measures again except for the multimodal MRI scans (Figure 2). The three study sites, the Beijing Hospital of Traditional Chinese Medicine, Guang'anmen Hospital, and Beijing Anding Hospital, will use the identical protocol to recruit patients. However, all clinical assessments, questionnaires, and bioassays will be conducted in the Beijing Hospital of Traditional Chinese Medicine and the multimodal MRI data be acquired at Guang'anmen Hospital. This study protocol is presented according to the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) guidelines.[40]

Power calculation

In a nonrandomized controlled trial by Rong et al. (2016), the treatment responsive was defined as 50% decrease relative to baseline in depression severity score (measured by HAM-D-24) [41]. The study revealed that 80% of patients treated with the taVNS and 39% of patients with the sham taVNS were responsive at the week 12 (Table 4 in Rong et al., 2016). [41] However, since there were no studies on the taVNS' effect in relapse prevention in remitted MDD, we were not able to determine clinically meaningful change in HAM-D-24 score. Therefore, the primary outcome measure of current study will be the rate of relapsed patients over one year (from baseline) period. In this study, we intend to recruit 45 subjects in each group with the consideration of about 20% drop out during follow up. In other word, 36 participants were used to estimate the power calculation. [42] Using the responsive rate to the taVNS (80%, p_1) and the sham taVNS (39%, p_2),

$$\mathrm{n} = \frac{2pq\,(Z_\alpha + Z_\beta)^2}{(p_1 - p_2)^2}$$

$p = \frac{p_1 + p_2}{2}$, $q = 1 - p$, $Z_{1-\frac{\alpha}{2}} = 1.96$ when alpha error (α) = 0.05, $p_1 = 0.8$, $p_2 = 0.39$, n = 36, so $Z_\beta =$ 1.5834, and β = 0.1133. We will have 88.67% (1-β) power to test the effect size of 0.41 $(p_1 - p_2)$ between the two groups based on the two-sample t-test at a significance level of 0.05.

Inclusion criteria

All patients will meet the following criteria: (1) ages between 18 and 60 years; (2) right-handed; (3) history of remitted recurrent MDD, implying more than two previous depressive episodes as assessed using the DSM-IV structured clinical interview and are in a remitted state (≥8 weeks assessed by the 17-item HAM-D ≤ 7);[4] (4) no history of neurologic or other chronic medical diseases; (5) no history of other psychiatric disorders such as schizophrenia or obsessive-compulsive disorder; (6) no history of

stimulant use for MDD; and (7) no history of alcohol or substance abuse.

Exclusion criteria

The exclusion criteria will be as follows: 1) ongoing addiction to drugs and alcohol; 2) previous head injury; 3) a family history of psychiatric illness; 4) obvious mental retardation (Mini-Mental State Examination ≥ 27) or dementia; 5) current pregnancy or breastfeeding; 6) any contraindications to an MRI scan; 7) failure to agree to signing the consent form.

Patient and public involvement

Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Ethics

We will follow according to the Declaration of Helsinki principles (Seoul, October 2008) to conduct this study. The study protocol was approved by the Medical Ethical Committee of Beijing Hospital of Traditional Chinese Medicine on January 18, 2019 (2018BL-076). Of note, at the request of the Beijing Medical Ethics Review Mutual Recognition Alliance (an organization that standardized of ethics committee behavior of its member hospital) for multicenter trial, the ethic approval obtained in one of the member hospitals (Beijing Hospital of Traditional Chinese Medicine in this study) can be used in the else of member hospitals, e.g., Guang'anmen Hospital and Beijing Anding Hospital. Before conducting this study, we had shown this ethical approval for the last two hospitals' ethics committees and got their approval for this multicenter trial. Written informed consent will be obtained from each participant. If desired, we will give participants up to 1 week to consider their decision. All investigators will receive good clinical practice training. We will anonymize and encrypt the raw data. Only researchers directly involved in the study will have access to data.

Measures

First, demographic information will be compiled for study participants, such as gender, age, marital status, education, contact information, etc. Second, the relevant epidemiological data will also be collected, including smoking, drinking, substance

abuse, family history of mental illness, age of first onset, duration of the first episode, number of previous depressive episodes, illness duration, changes in appetite, rhythm of life, and dosage and duration of medication.

Cognitive assessments

We will evaluate neurocognitive function, including memory, attention, processing speed, and executive function, using the Cambridge Neuropsychological Test Automatic Battery, Trail Making Test, and Wisconsin Card Sorting Test.[43]

Affective neuropsychological assessments

The 17-item HAM-D will be used to assess the severity of patient depression and the HAM-A to measure anxiety.[44] The Rumination Response Scale will be used to measure the severity of rumination symptoms and the Dysfunctional Attitude Scale will be used to measure the intensity of dysfunctional attitudes.[45] Each questionnaire will be completed on the day of scanning, at the end of taVNS treatment, and at the last day of 1-year follow up, respectively.

Blood measures

Pro-inflammatory cytokines

Fasting peripheral venous blood samples (5 mL) will be collected in tubes treated with ethylenediaminetetraacetic acid (EDTA) (S-Monovette, Sarstedt, Nümbrecht, Germany) at 08:00 by venipuncture. Plasma will be immediately separated by centrifugation (2000 *g*, 10 min, 4℃) and stored at -80℃ until analysis. IL-1, IL-6, IL-8 and TNF-α concentrations will be measured by the enzyme-linked immunosorbent assay (ELISA) (Human Quantikine ELISA, R&D Systems, Minneapolis, MN, USA) according to the manufacturer's protocols. Assay sensitivity will be 0.70 pg/ml.

Monoamine neurotransmitters

Blood samples of all participants will be collected at the three time-points (baseline, after treatment, and end of follow up). We will draw 10 mL of antecubital vein blood from each participant from the left arm and collect it in vacutainer tubes containing 0.5 mM EDTA. The whole blood samples will be fractionated by centrifugation at 2000 r/min for 10 min at room temperature as soon as they are delivered to the laboratory.

After centrifugation, serum will be separated into the upper layer and then individually transferred to a clean tube. All serum samples will be immediately stored at −80°C until analysis. The dopamine concentration will be determined using the high-performance liquid chromatographic (HPLC) method with the Acclaim HPLC (Bio-Rad, USA).[46] Serotonin concentration will be determined by HPLC with electrochemical detection, utilizing an internal standard (N-methyl-5HT).[47]

Salivary cortisol for HPA axis markers

Participants will also provide a saliva sample to assess the activity of the HPA axis before the MRI scan. Eating, drinking, smoking, or brushing teeth in the previous 15 min will not be allowed. We will instruct participants to provide five saliva samples over a day (at awakening, 30, 45, and 60 min thereafter, followed by a fifth measurement at 22:00 h) to reflect the diurnal morning awakening curve and evening HPA-axis activity. Salivette® (Sarstedt AG & Co., Nümbrecht, Germany) containers will be used to contain the saliva samples. After receipt, Salivette containers will be stored at -20ºC and later sent to centrifugation (3000 rpm for 5 min) and aliquotation, after which they will be frozen at -20ºC until analysis by ELISA (IBL International, Hamburg, Germany) to determine salivary cortisol levels.[48]

Change in 17-item HAM-D scores for MDD is the primary outcome parameter, change in cognition scales and bioassays are the secondary outcome parameters.

Multimodal MRI scanning procedure

T1-weighted sagittal high-resolution structural images, resting-state functional MRI (rs-fMRI), and diffusion tensor images (DTI) will be acquired in this study. See the online supplement for full details.

Blinding and randomization

After completion of baseline assessments, a research assistant will open an opaque envelope to determine the participant's random assignment (either taVNS or sham taVNS) without notifying the participants. Consistent with publications in the literature [49-51], randomization will be based on the random numbers generated from a random number table. This research assistant will not access the clinical assessments and MRI

related information. In addition, the researchers who are responsible for the participants' enrollment, clinical assessments, and intervention trainings will be blinded from participant' assessments.

Interventions

After MRI scanning, all participants will be trained to apply taVNS or sham taVNS.

A neural anatomy study showed that the innervation of the auricular branch of the vagus nerve is mainly distributed on the concha (including the outer auditory canal) and lower half of the back ear [52]. Thus, these areas should be the target of taVNS [53]. We used the taVNS therapeutic instrument, which is manufactured by Suzhou Medical Instruments Factory Co., Ltd. The stimulating pole part is an adjustable device, which can adjust the stimulating pole to the appearance of the patient's ear to ensure that the anatomy of the concha with the ear is in better apposition. When the participants enrolled in the study, the stimulation device will not change after being adjusted by the trainers. The trainers from our research team will show the study participants how to apply the taVNS, including the stimulation location and parameters settings. The participants can only finish the training when they master taVNS device well as approved by the trainers [41]. All subsequent interventions will be self-administered by the patients at home.[54] The taVNS treatment will be terminated when participants experienced intolerable symptoms, e.g., pain at stimulus points, dizziness, etc.

Treatment adherence

According to the latest international consensus, adherence should be recorded and analyzed in taVNS trials.[55] To enhance compliance, we will require all patients to complete daily diary entries, including the start time of every interference, details of side effects and improvement. In addition, we will check all the diaries through regular assessments and offer both telephone and face-to-face advisory sessions weekly during the entire treatment period[41]. Once non-compliance affects the analysis, e.g., the continuous interruptions of the taVNS intervention were longer than one week or the total duration of missing treatment was longer than 5 weeks (80%) [56], we will discuss and make a conservative decision (e.g., exclusion).

Real taVNS group

Location: The points for taVNS are in the auricular concha area where there are rich vagus nerve branch distributions. taVNS will be applied to the concha area of both ears simultaneously during treatment.

Intervention procedure: Patients will take a seated position or lie on their sides. After the stimulation points are disinfected according to standard practice, ear clips will be attached to the ear area (auricular concha) at the stimulation site. Stimulation parameters will include: 1) density wave adjusted to 20 Hz with a wave width less than 1 ms and 2) intensity adjusted based on the tolerance of the patient (4–6 mA). Each stimulation will last for 30 min and be completed twice a day (once in the morning and once again in the evening) [41]. The treatment will last for 5 days each week with 2 days off.

Sham taVNS group

Location: The stimulation points for sham taVNS are located at the superior scapha (outer ear margin midpoint) where there is no vagus nerve distribution. Similar to taVNS, sham taVNS will be applied on both ears simultaneously during the treatment. Stimulation at the superior scapha (outer ear margin midpoint) was regarded as a sham stimulation point, since it is relatively free of the vagus nerve distribution [52]. The same region was chosen for sham taVNS in other studies[41] [57]. Hein et al. demonstrated a significant reduction in beck depression inventory self-rating scores in the taVNS groups, compared to the sham group. However, HAM-D scores did not show significant reductions in both groups[57]. Further, Rong et al. also found a significant decrease of the HAM-D scores in the sham group at week 4 relative to baseline, but the reduction was significantly lower than that in the real taVNS group [41]. While the positive effect in the sham taVNS group may be caused by the stimulation regime, it may also be related to a placebo effect similar to the findings in antidepressants and psychotherapy study trials [28]. This is also one of the reasons that we choose the 6-month intervention to increase the significant differences in effects between the taVNS and shame taVNS.

Follow-up procedure

We will follow up the remitted recurrent MDD participants every 3 months and repeat the baseline measurements (except the multimodal MRI scan). To maximize recurrence detection rates, we will also instruct participants to contact us if recurrence occurs. The MDD relapse was confirmed by the study psychiatrist, and defined as clinical worsening and HAM-D > 15 [58]. We will inform the study participants of their clinical data and the treatments that participants received until the end of the follow-up.

For the prospective missing data during the study, it is mainly divided into two parts: the participant side (withdrew from the study unexpectedly) and the researcher side (e.g., failure of blood sample preservation or analyses, or data quality issues). In addition to the requirements of daily dairies for the study participants, we will also send them reminders (through automatic calls or emails) every day to reduce the prospective missing, and if necessary and possible, we will recruit extra participants to meet the requirements of this study. All the blood samples will be centrifugated and measured in time. The study participants will be trained before MRI so that they can keep their head still inside the MRI scanner to reduce head motion contamination to the data. Finally, all digital information e.g., clinical scales, bioassays results, and multimodal brain image data will be stored in an encrypted computer with double backups.

Data management

All the affective neuropsychology, cognition, pro-inflammatory cytokines, serum monoamine neurotransmitters, endocrinology, and multimodal neuroimaging data will be anonymized and upload on dedicated servers (http://www.bjzhongyi.com/) within six months after the trail complete.

MRI data preprocessing

See the online supplement for full details.

Distributions and missing data

First, we will inspect distributions and remove outliers and data non-compliant, such as saliva samples that significantly exceed the time limit. Second, we will transform non-normally distributed data where possible, otherwise, we will apply non-parametric tests if applicable. Third, missing data, including bioassay parameters caused by unexpected broken test tubes and the clinical scale loss accidentally could be solved with complete and available case analyses or multiple imputations [59 60]. Fourth, we will discard it once the multimodal imaging date is missing.

Statistical analysis plan

Statistical analysis will be performed using SPSS 22 Software (SPSS Inc., Chicago, IL, USA). The statistical tests will be two-sided with 5% significance level. Means and standard deviations will be used for the statistical description of continuous variables. For group analysis of each bioassay, Shapiro-Wilkes tests of distribution normality will first be performed, and those with non-Gaussian distributions will be either log-transformed or analyzed using non-parametric tests. A two-sample t-test and a chi-square test will be applied to compare the baseline characteristics of the participants between groups. For longitudinal data, different statistical methods were applied for different purpose. For hypothesis one, the paired *t* test will be applied to detect the difference of 17-item HAM-D scores of each group for baseline *vs.* treatment, as well as other index. For hypothesis two, the analysis of covariance (ANCOVA) will be used for the comparison of each period and each group. Bonferroni correction ($P < 0.05/3$) will be performed to compare every two different periods as a post hoc test if the variance analysis test result is significant. Finally, we will compute correlations between these neural function imaging indicators, cognitive scores, neuropsychological scores, levels of pro-inflammatory cytokines, monoamine neurotransmitters, and salivary cortisol.

Benefits and risk assessment

We focused on depressed patients in remission, who do not require antidepressant treatment measures but at risk of episode. Some of the participants will benefit more or

less from this study to reduce potential recurrence. In addition, the advantage of follow-up is that the recurrence of MDD can be detected timely, so as to provide timely psychiatric treatment. In case of relapse during the follow up period, a study psychiatrist will debrief the patient and provide appropriate treatment as needed. In case of suicide ideation or attempts, the study psychiatrist will referral the most appropriate emergency service.

Compensation

In addition to travel cost compensation, participants will receive ￥200. For completion of a follow-up scan we will pay ￥100.

Ethics and dissemination

The study protocol was approved by the Medical Ethical Committee of Beijing Hospital of Traditional Chinese Medicine on January 18, 2019 (2018BL-076). The trial is registered at the Chinese Clinical Trial Registry (www.chictr.org.cn, ChiCTR1900022618). The trial results will be published in peer-reviewed journals and at conferences.

Discussion

Summary

In summary, the current study will investigate the efficiency of taVNS in preventing MDD relapse and its mechanisms, focusing on multidimensions, e.g., brain circuits, inflammation status, monoamine neurotransmitters, and endocrine (glucocorticoids for HPA axis status), by comparing real versus sham taVNS intervention. We also examine the relationship between the change in depressive symptoms and the above multidimensional parameters, to determine predictive biomarker(s). The cohort of recurrent MDD participants will be followed up to test to what extent baseline measurements are predictive and/or how they change prospectively before recurrence. This will help elucidate the neural mechanisms underlying taVNS prevention of MDD relapse and open up the possibility of targeting novel therapeutic strategies that provide

a safe and effective method with fewer side effects for the prevention of MDD relapse. The durations of taVNS for treating active depression varied [55] from 0.5 months [57], 1 month [28], and 3 months [32 41]. Unfortunately, there are no existing studies examining the effect of taVNS in relapse prevention for remitted MDD. Therefore, we plan to use stimulation parameters based on the studies in mild depression in the literature. Rong et al reported that the effect size of 3-month taVNS in mild (HAM-D-24<20) and moderate (HAM-D-24>20) MDD was 0.4 and 0.68 respectively, suggesting that a stronger or longer treatment duration is needed for a stronger effect in mild MDD. Literature regarding the stimulus strength variation is scarce [41]. The current strength 4-6mA is regularly used and adjusted based on subjects' tolerance [61]. Only one review paper indicated that low-frequency stimulation (2–10 Hz) was not as efficient as higher frequency stimulation (20–30 Hz) [53]. Therefore, we will use the standard stimulation strength (4-6 mA continuous sinusoidal wave) and frequency (20Hz) as suggested in the literature [41 62].

Given that we won't increase stimulus strength, we've decided to extend the treatment duration, at least longer than 3 months, to get a moderate effect size for relapse prevention. In a recent meta-analysis, the risk of relapse was estimated to be around 40% in a 6-month period after electroconvulsive treatment [63]. In another prevention study, treatment with nortriptyline and the combination of nortriptyline and lithium were compared in preventing post-ECT relapse. The risk of relapse was 60% for nortriptyline and 39% for the combination of nortriptyline and lithium respectively, over a 6-month period [64]. Therefore, in our relapse prevention study, the taVNS/sham taVNS will be administered for 6 months and patients will be followed-up for another 6 months post treatment.

Limitations

This present study has several limitations. First, taVNS is a self-administered treatment whereby patient compliance may influence clinical outcome. Daily diary entries by

patients and our regular follow-up can enhance compliance. Despite this potential limitation, this kind of self-administered therapy provides a good choice for patients because of its feasibility and efficacy, and it also significantly reduces treatment expenses and time costs. Second, to overcome the potential confounding effects of antidepressants and other psychotropic medication, only participants who currently do not use these drugs will be included. Third, due to limited budget, we just do MRI scans twice. In the future, we will apply for more grants so as to repeat MRI scans assessment at 1y-follow up. Fourth, the gap between study compensation and participants' actual expenditures did cause the recruitment bias. Of note, we will not include single-episode MDD participants.

Trial status

This study protocol was approved by the Medical Ethical Committee of the Beijing TCM Hospital on January 18, 2019 (authorization 2018BL-076), as well as Guang'anmen Hospital and Beijing Anding Hospital. This trial has been registered since April 19, 2019 (Registration Number ChiCTR1900022618). The trial started on May 11, 2019. The first participant was studied on May 15, 2019, and 60 participants have been recruited as of the date of this submission. The trial is currently recruiting participants. We predict that recruitment will be completed by October 2021.

Author Contributions

CHL and LW contributed to the conception of the study. The protocol was drafted by ZQZ and was reviewed by LW and CHL. ZQZ, ZPG and XXW will supervise the trial. XXW was applied for the ethical approval. PS provided the guidance of statistical analysis. ZQZ and ZPG designed and drew the figures. All authors reviewed and approved the publication of the protocol.

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Competing interests statement

The authors declare that they have no conflict of interests.

Figure legends

Figure 1. Theoretical framework. The blue part of Figure 1 illustrates the main theories of major depressive disorder (MDD) related to the hypothalamic-pituitary-adrenal (HPA) axis, pro-inflammatory cytokines, and monoamine neurotransmitters. The red part of Figure 1 shows the possible mechanism of taVNS in preventing MDD recurrence. It is proposed that stress, chronic inflammation, and weakening of the immune system induce activation of the HPA axis, and that impaired negative feedback induces the sustained rise of glucocorticoids and its resistant, then promotes the elevation of pro-inflammatory cytokines. Furthermore, it is proposed that some peripheral pro-inflammatory cytokines can act on neurons and supporting cells to not only cross the blood-brain barrier directly (e.g., astrocytes and microglia), but also elicit depressive-like behaviors through afferent signals pathways (e.g., glucocorticoid receptors and monoamine neurotransmitters). taVNS may inhibit MDD episodes by affecting brain circuits and reducing inflammation through effects on pro-inflammatory cytokines.

Figure 2. Study design. Figure 2 depicts the design of the present study. The enrolled patients will participate in the initial assessments for depression, anxiety, and other clinical variables. The baseline data will also include neuropsychological tests as well as blood and saliva related measures. Subsequently, participants will have a magnetic resonance imaging (MRI) session for structural (T1-weighted and diffuse tensor imaging) and resting-state functional MRI scans. Then, we will assign participants randomly into the real and sham taVNS groups without their awareness. After the 6-month intervention, we will repeat all measures conducted at the baseline. Next, we will follow up patients clinically for another 12 months and assess their severity of depression and anxiety to detect relapse. At the end of the 12-month follow-up, we will repeat the measures as the baseline again (e.g., HAM-D, HAM-A, neuropsychological tests, blood, and saliva samples related measures).

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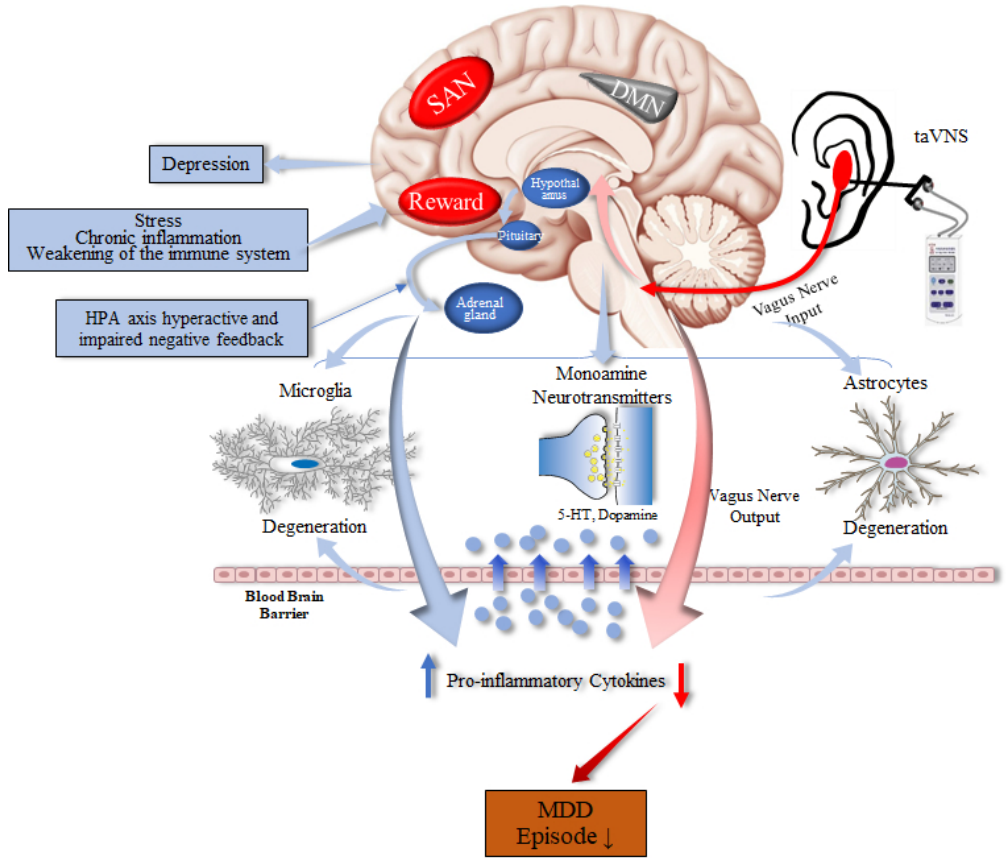

Figure 1 Theoretical framework. The blue part of Figure 1 illustrates the main theories of major depressive disorder (MDD) related to the hypothalamic-pituitary-adrenal (HPA) axis, pro-inflammatory cytokines, and monoamine neurotransmitters. The red part of Figure 1 shows the possible mechanism of taVNS in preventing MDD recurrence. It is proposed that stress, chronic inflammation, and weakening of the immune system induce activation of the HPA axis, and that impaired negative feedback induces the sustained rise of glucocorticoids and its resistant, then promotes the elevation of pro-inflammatory cytokines. Furthermore, it is proposed that some peripheral pro-inflammatory cytokines can act on neurons and supporting cells to not only cross the blood-brain barrier directly (e.g., astrocytes and microglia), but also elicit depressive-like behaviors through afferent signals pathways (e.g., glucocorticoid receptors and monoamine neurotransmitters). taVNS may inhibit MDD episodes by affecting brain circuits and reducing inflammation through effects on pro-inflammatory cytokines.

64x55mm (300 x 300 DPI)

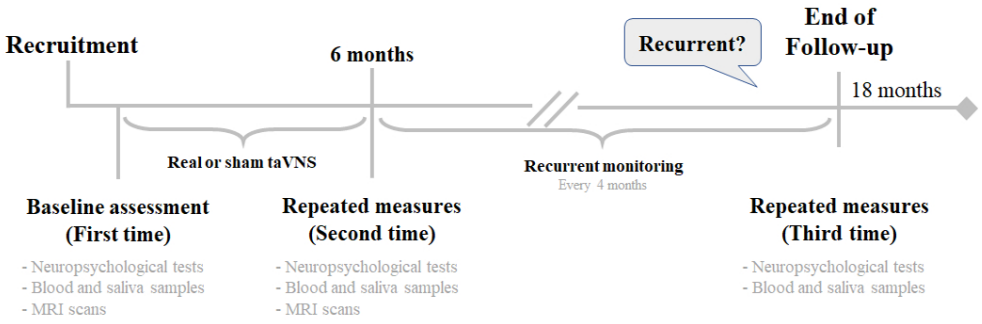

Figure 2. Study design. Figure 2 depicts the design of the present study. The enrolled patients will participate in the initial assessments for depression, anxiety, and other clinical variables. The baseline data will also include neuropsychological tests as well as blood and saliva related measures. Subsequently, participants will have a magnetic resonance imaging (MRI) session for structural (T1-weighted and diffuse tensor imaging) and resting-state functional MRI scans. Then, we will assign participants randomly into the real and sham taVNS groups without their awareness. After the 6-month intervention, we will repeat all measures conducted at the baseline. Next, we will follow up patients clinically for another 12 months and assess their severity of depression and anxiety to detect relapse. At the end of the 12-month follow-up, we will repeat the measures as the baseline again (e.g., HAM-D, HAM-A, neuropsychological tests, blood, and saliva samples related measures).

90x90mm (300 x 300 DPI)

Multimodal MRI scanning procedure

Acquisition of MRI brain imaging data will be conducted using a Siemens Skyra 3-Tesla scanner (Siemens, Erlangen, Germany) in Guang'anmen Hospital, China Academy of Chinese Medical Sciences, Beijing. T1-weighted sagittal high-resolution structural images will be acquired with the three-dimensional fast spoiled gradient-echo sequence: repetition time/echo time (TR/TE), 2,530 ms/3.39 ms; thickness/gap, 1.0/0 mm; matrix, 256 × 256; voxel size, 1 × 1 × 1 mm^3; and 9° flip angle (FA). Resting-state functional MRI (rs-fMRI) encompassing the whole brain will be obtained using an echo-planar imaging sequence: TR/TE, 2000 ms/30 ms; 90° flip angle; matrix, 64 × 64; thickness/gap, 4.0 mm/0.6 mm, field of view (FOV), 232 × 232 mm^2; 8 min; paralleled by anterior commissure-posterior commissure line. All participants will receive the following instructions: stay awake, do not move, close your eyes, and do not think about anything. Diffusion tensor images (DTI) will be acquired via an echo-planar imaging sequence using the following parameters: TR/TE = 11000 ms/98 ms, FOV = 256 × 256 mm^2, matrix = 128 × 128, slice thickness = 2 mm and 60 continuous axial slices without a gap. The diffusion sensitizing gradients will be applied to 12 nonlinear directions (b = 1,000 s/mm^2), together with an acquisition image without diffusion weighting (b = 0 s/mm^2). For anatomic reference, T1 images will be obtained before resting-state scans.

MRI data preprocessing

The T1 data will be processed using Freesurfer (http://surfer.nmr.mgh.harvard.edu). The processing procedures include motion correction, skull stripping, segmentation of

white matter, creation of the pial surface and surface of the white/gray junction, inflation of the folding surface plane, and topology correction. All the above procedures will be completed automatically. Parameters such as global cortical structure, whole-brain cortical thickness, volume and surface area, and so on, will be obtained through this procedure. [1]

Resting-state functional MRI (rs-fMRI) data will be preprocessed using Data Processing and Analysis for Brain Imaging (DPABI, http://www.rfmri.org/dpabi) based on Statistical Parametric Mapping (SPM12, http://www.fil.ion.ucl.ac.uk/spm). The rs-fMRI data will undergo slice-timing correction, motion correction, scaling to percent signal change, smoothing with a Gaussian kernel of 6 mm full-width-at-half maximum, bandpass temporal filtering (0.01–0.1 Hz), and grand mean intensity normalized. We will evaluate the brain circuits through regional homogeneity (ReHo), amplitude of low-frequency fluctuations (ALFF), fractional ALFF (fALFF), functional connectivity, independent component analysis, and graph theoretical network analyses, and so on.

DTI data will be preprocessed using the Pipeline for Analyzing braiN Diffusion imAges (PANDA, https://www.nitrc.org/projects/panda/) based on Functional MRI of the Brain (FMRIB's) Software Library (FSL) tools (http://www.fmrib.ox.ac.uk/fsl). The preprocessing procedures include converting DICOM files into NIFTI images, estimating the brain mask, cropping the raw images, correcting for the eddy-current effect, averaging multiple acquisitions, calculating diffusion tensor (DT) metrics. The DT metrics include fractional anisotropy (FA), mean diffusivity, axial diffusivity, and radial diffusivity. The four DTI-metric images were then normalized to the MNI space

with a 1-mm spatial resolution template and outputted for further analysis. Several voxel-based analyses will be applied for the above four parameters to reflect the change of white matter microstructure. In addition, we will also conduct Tract-Based Spatial Statistics (TBSS) analysis to avoid false results caused by spatial smoothing. For TBSS analysis, all the aligned FA images will be skeletonized and a mean FA skeleton generated, then the individual images with data on the skeleton will be created for voxel-wise statistical analysis on the skeleton. Statistical analyses will be performed using nonparametric permutation testing (Randomize in FSL) with 5000 Monte Carlo simulations. We will evaluate the structural differences in each parameter above between the groups accounting for age and head motion.[2]

Reference

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Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet	1,2,15

		registered, name of intended registry	
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	N/A It's not a World Health Organization Trial Registration Data Set.
Protocol version	#3	Date and version identifier	1
Funding	#4	Sources and types of financial, material, and other support	17
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1 (Title Page)
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	N/A
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	N/A The sponsor and funder are not involved in this study.
Roles and	#5d	Composition, roles, and responsibilities of the	N/A

responsibilities: committees		coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3-5
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	4, 5
Objectives	#7	Specific objectives or hypotheses	5,6
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	6-7
Methods: Participants, interventions, and outcomes			

Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	6
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7-8
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	10-11
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	11
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	11
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	N/A There were no relevant

			concomitant care and interventions that are permitted or prohibited during the trial.
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	8-10
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Figure 2
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	7
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	11

Methods:			
Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	10
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	10
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	10
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	10

Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	13
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	8, 11, 13
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	11
Data management	#19	Plans for data entry, coding, security, and	13

		storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	14
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	N/A Additional statistical analyses will be conducted on a case-by-case basis after trial data collection is complete.
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	13-14
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting	N/A

		structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	The study will not have a formal DMC since adverse intervention events have not been reported.
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	11
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	N/A
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	N/A
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	N/A Because it has been approved by the ethic

			committee of Beijing Hospital of Traditional Chinese Medicine.
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	N/A There is no above corresponding plan by now.
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	8
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	8
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	N/A There were on conflict interests to others.

Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	10
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	N/A
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	N/A
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	8
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	Please refer to the supplementary Patient Consent Form

Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	N/A