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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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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 non-invasive, 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 multicentre, randomised, patient-blinded and evaluators double-blinded trial. We will randomise 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: proinflammatory 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 Hamilton Depression Rating Scale 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 18 January 2019 (2018BL-076). The trial results will be published in peer-reviewed journals and at conferences.

Trial registration number ChiCTR1900022618.

Strengths and limitations of this study

- This will be the first prospective, doubled-blinded, randomised controlled trial on transcutaneous vagus nerve stimulation (taVNS) to prevent the relapse of major depressive disorder (MDD).
- This study integrates six distinct types of measures from clinical symptoms, neuropsychological battery, inflammation, hypothalamic–pituitary–adrenal 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 characterised 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 WHO 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 patients with MDD 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-related or affective-related residual symptoms, for example, 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–behavioural 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 US 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 (ABVN), known as the vagally innervated external ear regions.[20] From a neuroanatomic view, the 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 (ACC), 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 (eg, 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 ACC (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 (eg, IL-1, IL-2 and IL-6) and tumour necrosis factor-α (TNF-α) can lead to depressive behavioural symptoms and changes in the course of MDD through various pathways.[36] Proinflammatory 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, are risk factors for the aetiology and pathophysiological mechanisms of MDD.[39] As a result, it is suggested that taVNS may affect the HPA axis, proinflammatory 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).

This 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 hypothesise 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 hypothesise that taVNS can significantly alter HPA-axis activity, reduce inflammation, increase levels of monoamine neurotransmitters (eg, 5-HT, DA), and change grey/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. This study will be conducted as a multicentre, prospective parallel-group,

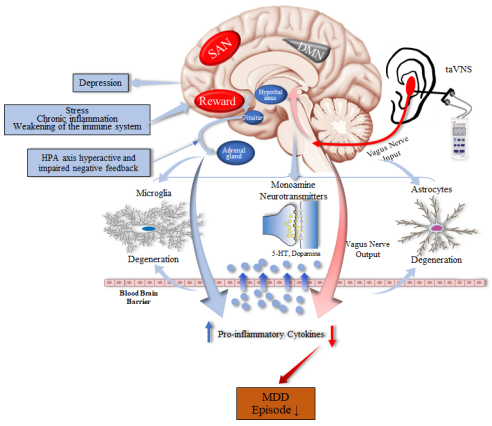

Figure 1 Theoretical framework. The blue part of figure 1 illustrates the main theories of major depressive disorder (MDD) related to the hypothalamic–pituitary–adrenal (HPA) axis, proinflammatory cytokines and monoamine neurotransmitters. The red part of figure 1 shows the possible mechanism of taVNS 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 proinflammatory cytokines. Furthermore, it is proposed that some peripheral proinflammatory cytokines can act on neurons and supporting cells to not only cross the blood–brain barrier directly (eg, astrocytes and microglia), but also elicit depressive-like behaviours through afferent signals pathways (eg, glucocorticoid receptors and monoamine neurotransmitters). taVNS may inhibit MDD episodes by affecting brain circuits (eg, default mode network (DMN), salience network (SAN) and reward network) and reducing inflammation through effects on proinflammatory cytokines. 5-HT, 5-hydroxytryptamine; taVNS, transcutaneous vagus nerve stimulation.

patient-assessor-blinded, randomised controlled trial, consisting of two stages. First, we will obtain baseline measures, including demographic information, neuropsychological scales, multimodal MRI scans, HPA axis markers, proinflammatory cytokines, and monoamine neurotransmitters. Second, 90 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

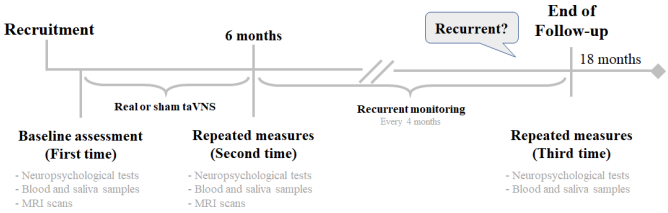

Figure 2 Study design. Figure 2 depicts the design of this study. The enrolled patients will participate in the initial assessments for depression, anxiety and other clinical variables. The baseline data will also include neuropsychological tests as well as blood and saliva-related measures. Subsequently, participants will have an 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 (eg, HAM-D, HAM-A, neuropsychological tests, blood and saliva samples related measures). HAM-D, Hamilton Depression Rating Scale; taVNS, transcutaneous vagus nerve stimulation.

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 guidelines.[40]

Power calculation

In a non-randomised controlled trial by Rong *et al*, 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 .[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 1 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),

$$n = \frac{2\bar{p}\bar{q}\left(Z_\alpha + Z_\beta\right)^2}{\left(p_1 - p_2\right)^2}$$

$\bar{p} = \frac{p_1+p_2}{2}$, $\bar{q} = 1 - \bar{p}$, $Z_{1-\frac{\alpha}{2}} = 1.96$when alpha error ($\alpha$)=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 Diagnostic and Statistical Manual, Fourth Edition (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 neurological 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 breast feeding; (6) any contraindications to an MRI scan and (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 18 January 2019 (2018BL-076). Of note, at the request of the Beijing Medical Ethics Review Mutual Recognition Alliance (an organisation that standardised of ethics committee behaviour of its member hospital) for multicentre 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, for example, 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 multicentre 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 anonymise 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

Proinflammatory cytokines

Fasting peripheral venous blood samples (5 mL) will be collected in tubes treated with EDTA (S-Monovette, Sarstedt, Nümbrecht, Germany) at 08:00 by venipuncture. Plasma will be immediately separated by centrifugation (2000 g, 10 min, 4°C) and stored at −80°C until analysis. IL-1, IL-6, IL-8 and TNF-α concentrations will be measured by the ELISA (Human Quantikine ELISA, R&D Systems, Minneapolis, Minnesota, 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 DA 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, utilising 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 hours) 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 −20°C and later sent to centrifugation (3000 rpm for 5 min) and aliquotation, after which they will be frozen at −20°C 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, and diffusion tensor images will be acquired in this study. See the online supplemental file 1 for full details.

Blinding and randomisation

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] randomisation 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 ABVN 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. 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, for example, pain at stimulus points, dizziness, etc.

Treatment adherence

According to the latest international consensus, adherence should be recorded and analysed 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, for example, 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 (eg, 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 with 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 maximise 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 (eg, 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, for example, 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, proinflammatory cytokines, serum monoamine neurotransmitters, endocrinology and multimodal neuroimaging data will be anonymised and upload on dedicated servers (http://www.bjzhongyi.com/) within 6 months after the trail complete.

MRI data preprocessing

See the online supplemental file 1 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 V.22 Software (SPSS). The statistical tests will be two sided with 5% significance level. Means and SD 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 analysed using non-parametric tests. A two-sample t-test and a χ^2 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 1, 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 2, the analysis of covariance 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 proinflammatory 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 18 January 2019 (2018BL-076). 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, for example, 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 (20 Hz) as suggested in the literature.[41 62]

Given that we will not 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 electroconvulsive therapy 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 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 18 January 2019 (authorisation 2018BL-076), as well as Guang'anmen Hospital and Beijing Anding Hospital. This trial has been registered since 19 April 2019. The trial started on 11 May 2019. The first participant was studied on 15 May 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.

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Contributors C-HL, X-YL, and LW contributed to the conception of the study. The protocol was drafted by Z-QZ and was reviewed by LW and C-HL. Z-QZ, X-YL, Z-PG and X-XW will supervise the trial. X-XW and X-Y L applied for the ethical approval. PS provided the guidance of statistical analysis. Z-QZ and Z-PG designed and drew the figures. All authors reviewed and approved the publication of the protocol.

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