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Transcutaneous Vagus Nerve Stimulation Modulates Default Mode Network in Major Depressive Disorder

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ABSTRACT

BACKGROUND: Depression is the most common form of mental disorder in community and health care settings and current treatments are far from satisfactory. Vagus nerve stimulation (VNS) is a Food and Drug Administration approved somatic treatment for treatment-resistant depression. However, the involvement of surgery has limited VNS only to patients who have failed to respond to multiple treatment options. Transcutaneous VNS (tVNS) is a relatively new, noninvasive VNS method based on the rationale that there is afferent/efferent vagus nerve distribution on the surface of the ear. The safe and low-cost characteristics of tVNS have the potential to significantly expand the clinical application of VNS.

METHODS: In this study, we investigated how tVNS can modulate the default mode network (DMN) functional connectivity (FC) in mild or moderate major depressive disorder (MDD) patients. Forty-nine MDD patients were recruited and received tVNS or sham tVNS (stVNS) treatments.

RESULTS: Thirty-four patients completed the study and were included in data analysis. After 1 month of tVNS treatment, the 24-item Hamilton Depression Rating Scale score reduced significantly in the tVNS group as compared with the stVNS group. The FC between the DMN and anterior insula and parahippocampus decreased; the FC between the DMN and precuneus and orbital prefrontal cortex increased compared with stVNS. All these FC increases are also associated with 24-item Hamilton Depression Rating Scale reduction.

CONCLUSIONS: tVNS can significantly modulate the DMN FC of MDD patients; our results provide insights to elucidate the brain mechanism of tVNS treatment for MDD patients.

Keywords: Default mode network, fMRI, Functional connectivity, Major depressive disorder, Transcutaneous vagus nerve stimulation, Vagus nerve stimulation

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Major depressive disorder (MDD) is the fourth leading cause of disability worldwide (1) and is projected to become the second leading cause of disability worldwide by the year 2020 (2,3). Despite the critical need, current treatments for this disorder are far from satisfactory due to high nonresponse rates to treatments, high relapse rates, and frequent intolerable side effects (1,3).

Vagus nerve stimulation (VNS) is a relatively new Food and Drug Administration approved somatic treatment for treatment-resistant depression that can produce significant and clinically meaningful antidepressant effects (1,4–6). However, the involvement of surgery, perioperative risks, and potentially significant side effects has limited this treatment to patients who have been treated for depression in the past but have failed to respond to at least four prescribed medications and/or established somatic treatment options such as electroconvulsive therapy for MDD (7).

To overcome the potential barriers of applying VNS, a noninvasive VNS method, transcutaneous vagus nerve stimulation (tVNS), has been developed. The rationale for using tVNS on the ear is based on anatomical studies that suggest that the ear is the only place on the surface of the human body where there is afferent vagus nerve distribution (8,9). Thus, direct stimulation of the afferent nerve fibers on the ear should produce an effect similar to classic VNS in reducing depressive symptoms but without the burden of surgical intervention (10,11). In past years, tVNS has been applied to treat MDD (10) and other disorders such as epilepsy (12,13), and prediabetes (14). Yet, the underlying mechanism of tVNS remains unclear.

In past decades, with the aid of brain imaging tools, accumulating evidence has demonstrated that MDD is associated with structural and functional abnormalities in brain circuits involved in emotional processing, self-representation, reward, and external stimulus (stress, distress) interactions (15–22); these brain regions include the hippocampus, amygdala, anterior cingulate cortex, and medial prefrontal cortex.

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Interestingly, these brain regions also fall within the default mode network (DMN), a brain network believed to be involved in self-referential processing, affective cognition, and emotion regulation (23,24).

Thus, the DMN functional connectivity (FC) has drawn investigators' attention in MDD research (25–29). Studies have found DMN functional connectivity changes in MDD patients (30–36), and these changes are associated with psychiatric measurements, such as rumination score, in MDD patients. For instance, studies have found increased functional connectivity of DMN with the subgenual anterior cingulate cortex in MDD patients (26,30). After transcranial magnetic stimulation treatment, the abnormal increase in functional connectivity of DMN and subgenual anterior cingulate cortex was reduced (34). These studies demonstrated that the FC of DMN can be a useful tool in understanding the underlying mechanism of MDD treatment.

In this study, we investigated the resting state functional connectivity (rsFC) changes after the longitudinal tVNS as compared with sham tVNS (stVNS) in patients with mild or moderate depressive symptoms. We hypothesize that the longitudinal tVNS may significantly modulate the rsFC of DMN and reduce symptoms in MDD patients.

METHODS AND MATERIALS

Study Population

Forty-nine patients with mild or moderate MDD were recruited for the trial. ICD-10 classification of mental and behavioral disorders was used for diagnosis of MDD. Patients who voluntarily provided informed consent and met inclusion/exclusion criteria were enrolled in this study.

Inclusion criteria included the following: 1) patient meets ICD-10 diagnosis standard: mild (two typical + two other core symptoms) or moderate (two typical + three other core symptoms); 2) patient is 16 to 70 years of age; 3) patient stopped taking antidepressive medication or other psychiatric medications 2 weeks before the intervention started; 4) patient has a junior high school education and can understand the scales; and 5) patient has exhibited symptoms for at least 2 months but no longer than 2 years.

Exclusion criteria included the following: 1) patients with current addiction to drugs; 2) patients with severe depression or suicidal thoughts; 3) patients with other severe organic diseases, such as severe heart disease, kidney failure, etc; and 4) patients who did not agree to sign the consent form.

Recruitment Procedures

All patients were recruited using advertisements and by sending flyers to the four hospitals involved in the study. In this study, tVNS was the only treatment the patients received. Due to safety concerns and to increase the homogeneity of the study, we decided to include only patients with mild or moderate depressive symptoms. After passing a prescreening, potentially eligible patients provided informed consent in the presence of a study physician.

We used a single-blinded clinical trial to investigate the antidepressant effects of solo tVNS treatment. The first cohort received tVNS. After demonstrating the effect of tVNS, we recruited the second cohort of patients who received 4 weeks of sham tVNS as a control in this study.

Intervention

After screening, all patients were trained to apply the tVNS or stVNS. All subsequent treatments were self-administered by the patients at home. Patients were also instructed to complete a patient diary booklet each day to describe any side effects corresponding with or temporally related to treatment. The investigators checked all booklets at the end of 4-week treatment. All procedures performed in the stVNS treatment group were identical to the procedures for the tVNS group.

tVNS Treatment

Location. The points for tVNS are located in the auricular concha area where there are rich vagus nerve branch distributions (Figure 1).

Intervention Procedure. Patients took a seated position or laid on their side. After the stimulation points were disinfected according to standard practice, ear clips were attached to the ear area (auricular concha) at the stimulation site. Stimulation parameters included the following: 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 treatment lasted 30 minutes and was carried out twice a day, at least 5 days per week, for the duration of the treatment period (4 weeks).

stVNS Treatment

Location. The stimulation points for stVNS are located at the superior scapha (outer ear margin midpoint) where there is no vagus nerve distribution (Figure 1).

Since all treatments were self-administered by the MDD patients, all patients were required to complete daily entries in a diary that was checked during assessments (at the end of week 4) to enhance compliance.

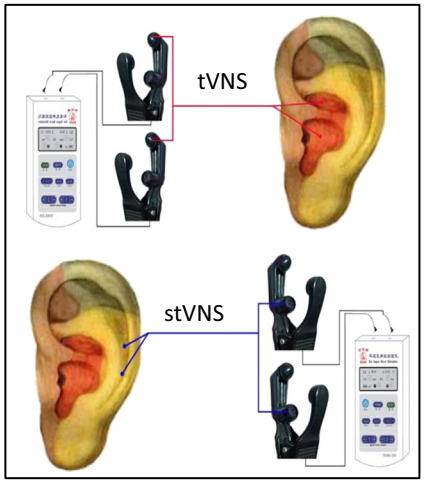

Figure 1. Locations of the stimulation electrodes on the auricular surface for transcutaneous vagus nerve stimulation (tVNS) and sham transcutaneous vagus nerve stimulation (stVNS).

Clinical Outcomes and Statistical Analysis

All end points were measured at week 0 and week 4. The primary end point was the 24-item Hamilton Depression Rating Scale (HAM-D). The secondary end points included the Hamilton Anxiety Rating Scale (HAM-A), Self-Rating Anxiety Scale (SAS), and Self-Rating Depression Scale (SDS).

Our analyses were based on the intention-to-treat principle. Statistical analysis was performed using SPSS 19.0 Software (SPSS Inc., Chicago, Illinois). Repeated measurements were applied to compare primary and secondary outcomes. Age and gender were also included in the model as covariates.

Functional Magnetic Resonance Imaging Data Acquisition and Analysis

Each subject participated in identical functional magnetic resonance imaging (fMRI) scanning sessions before and after 1 month of treatment. The fMRI brain imaging acquisition was conducted on a 1.5 Tesla GE Signa MRI system (GE Healthcare, Buckinghamshire, United Kingdom) equipped with the standard two-channel birdcage head coil. T1-weighted high-resolution structural images were acquired with the three-dimensional fast spoiled gradient-echo sequence (matrix 192 $\times$ 256, field of view [FOV] 200 mm, flip angle 15°, slice thickness 1.4 mm). T2-weighted functional images encompassing the whole brain were acquired with the gradient echo echo-planar imaging sequence (echo time 30 ms, repetition time 2500 ms, matrix 64 $\times$ 64, FOV 240 mm, flip angle 90°, slice thickness 3.0 mm, gap .5 mm, 41 slices, paralleled by anterior commissure-posterior commissure line). Image collection was preceded by four dummy scans to allow for equilibration of the MRI signal. Two 6-minute resting state fMRI scans were applied while the subjects were required to keep their eyes closed.

We analyzed pretreatment and posttreatment resting state data using independent component analysis (ICA) in the FMRIB (Functional, Structural and Diffusion MRI brain software library, Oxford, United Kingdom) (37–40). We first applied a bandpass filter between .01 Hz and .1 Hz to the functional time series and then corrected for motion using MCFLIRT. We corrected slice timing and stripped the skull using the Brain Extraction Tool and smoothed it (full width at half maximum = 5 mm). Next, we registered the functional data to their respective skull-stripped anatomical volume and further registered it to the Montreal Neurological Institute 152 stereotactic template using linear affine transformations with 12 degrees of freedom. We then concatenated the functional data into four-dimensional data and performed a probabilistic independent component analysis using multivariate exploratory linear optimized decomposition into independent components (41) on the dataset to identify 20 resting state networks. Using an algorithm, we searched for similarities between the default mode network in our group-level networks and the template networks derived from 1414 healthy subjects to identify the corresponding network for our results (38).

Then, dual regression was applied (38). Using the previously defined DMN as spatial regressors in a general linear model (GLM), we were able to extract the temporal dynamics associated with each spatial map. The resulting time courses served as temporal regressors in a GLM to generate subject-specific maps of the whole brain for each subject. Finally, group analyses were performed using the whole-brain subject-specific network maps from the second GLM. The results represent the strength of FC for each voxel within the DMN.

To investigate the modulation effect of tVNS treatment, we compared the changes after treatment (posttreatment − pretreatment) in the tVNS group with that of the stVNS group. To explore the association between the clinical outcomes and FC changes, we performed regression analyses using the network connectivity change maps between posttreatment and pretreatment and corresponding changes in clinical outcomes. For both analyses, age and gender were included in the model as noninterest covariates. To further control the potential residual effect of head motion, we also included average head motion difference (posttreatment − pretreatment) for each individual as covariate during analysis (42). A threshold of voxelwise $Z > 2.6$ and a corrected cluster significance threshold of $p < .05$ with more than 20 contiguous voxels were applied in data analysis.

RESULTS

Out of 49 subjects (27 in the VNS group, 22 in the control group) recruited into the study, 35 patients (19 in VNS group, 16 in control group) completed the two fMRI scans (0 and 4 weeks). Out of eight patients who dropped out in the VNS group, four dropped due to loss of contact, three due to disinterest, and one due to a scheduling conflict. Of the seven patients who dropped out in the stVNS group, three dropped due to scheduling conflicts, three due to disinterest, and one due to poor data quality. There was no statistical difference in dropout rate between the two groups.

Of 35 subjects who completed the study, we excluded one additional subject in the tVNS group due to large head motion during data analysis. Of the 34 patients left for data analysis, there was no significant difference on head motion pretreatment and posttreatment between the two groups ($p = .89$ and $p = .95$ between the tVNS and stVNS groups using two-sample t test).

Baseline characteristics of the two groups are shown in Table S1 in Supplement 1. There was no significant difference between the two groups in age, gender, HAM-D, HAM-A, SDS, and SAS at the beginning of the treatment.

The clinical outcome measurements of pretreatment and posttreatment are shown in Table 1. Repeated measurements show that there was significant interaction between the treatment group (tVNS and stVNS) and treatment time (pretreatment and posttreatment) on all measured clinical outcomes (HAM-D: $p = .002$; SAS: $p = .013$; SDS: $p = .012$) except HAM-A, which showed a marginal significant difference ($p = .053$). In addition, we also found a significant main effect of treatment time point (pretreatment > posttreatment) in SDS ($p = .019$).

fMRI Result

The DMN obtained from ICA analysis is shown in Figure S1 in Supplement 1. The network is consistent with previously published results (38). Brain regions observed in the network include the medial prefrontal cortex (mPFC), anterior cingulate cortex (ACC), precuneus, posterior cingulate cortex, and the bilateral parietal cortex.

Table 1. Clinical Outcome Measurements in Each Group (Pretreatment and Posttreatment)

	tVNS Group		stVNS Group	
Items	Pretreatment	Posttreatment	Pretreatment	Posttreatment
HAM-D[a]	28.50 ± 6.42	15.00 ± 4.59	28.56 ± 4.79	23.00 ± 4.68
HAM-A	19.61 ± 5.88	11.67 ± 7.06	16.88 ± 4.19	13.44 ± 3.71
SAS Score[a]	56.56 ± 8.69	42.83 ± 10.90	58.50 ± 10.71	54.44 ± 5.72
SDS Score[a]	66.33 ± 11.11	50.56 ± 12.05	66.94 ± 9.28	61.19 ± 10.02

HAM-A, Hamilton Anxiety Rating Scale; HAM-D, Hamilton Depression Rating Scale; SAS, Self-Rating Anxiety Scale; SDS, Self-Rating Depression Scale; stVNS, sham transcutaneous vagus nerve stimulation; tVNS, transcutaneous vagus nerve stimulation.

[a]Indicates significant difference in pretreatment and posttreatment changes ($p < .05$) using repeated measurements after adjusting for age and gender.

The comparison on the posttreatment and pretreatment differences between the two groups showed that after treatment, patients in the tVNS group, as compared with patients in the stVNS group (tVNS > stVNS), showed significant FC differences between the DMN and the following regions: the bilateral fusiform gyrus and thalamus; the left orbital prefrontal cortex (OPFC), precuneus, and temporoparietal junction; the right superior prefrontal cortex, dorsal lateral prefrontal cortex, middle temporal gyrus, and parahippocampus (Figure 2; Table S2 in Supplement 1).

The opposite comparison (stVNS > tVNS) showed significant FC differences between the DMN and the bilateral operculum and occipital cortex; left mPFC, superior temporal gyrus, postcentral gyrus, and occipitotemporal gyrus; and right insula, parahippocampus/hippocampus, and inferior parietal lobule (Figure 3; Table S2 in Supplement 1).

Regression analysis showed a positive association between the depression severity change as measured by HAM-D (posttreatment vs. pretreatment) score and the corresponding FC changes between the DMN and the left OPFC, middle occipital gyrus, right operculum, anterior insula, dorsal anterior cingulate cortex, inferior parietal lobule, inferior temporal gyrus, and cuneus (Figure 2; Table S3 in Supplement 1). The analysis showed a negative association between the depression severity change as measured by HAM-D (posttreatment vs. pretreatment) and the corresponding FC changes between the DMN and the bilateral dorsal lateral prefrontal cortex, fusiform, left mPFC, operculum, insula, temporoparietal junction, the right OPFC, rostral anterior cingulate cortex, precuneus, the middle temporal gyrus, and parahippocampus (Figure 3; Table S3 in Supplement 1). The brain regions of the OPFC and precuneus overlap with the contrast comparing tVNS and stVNS (tVNS > stVNS) (Figure 3).

DISCUSSION

In this study, we investigated the DMN rsFC changes before and after 1 month of tVNS treatment as compared with stVNS. The results showed that the HAM-D and other clinical outcomes significantly decreased in the tVNS group as compared with stVNS. Further, we found tVNS can significantly modulate the rsFC of DMN in brain regions associated with emotion and affect. The rsFC changes in the DMN are also significantly associated with the MDD severity changes as indicated by HAM-D score, implying that tVNS may achieve the treatment effect by modulating the FC of the DMN.

In a previous pilot study, Hein *et al.* (10) investigated the short-term (2 weeks) therapeutic effect of transcutaneous auricular VNS on patients suffering from major depression using an add-on design. They found that transcutaneous auricular VNS can significantly reduce the Beck Depression Inventory as compared with the sham condition. But there was no significant difference on the HAM-D. In this study, tVNS could not only reduce HAM-D and SDS but also reduce the anxiety symptoms in these patients as indicated by SAS and HAM-A (marginally significant) in comparison with stVNS at week 4. These results suggest that tVNS may also be extended to anxiety disorders in the future.

Interestingly, we found that the symptoms in the stVNS group also showed a significant decrease after treatment (HAM-D: $p = .002$, HAM-A: $p = .001$, SDS: $p = .03$) using paired t test to compare pretreatment and posttreatment outcomes. We speculate that this reflects the placebo effect produced by stVNS. The large placebo effect observed in our study is consistent with findings from previous clinical trials testing the efficacy of antidepressants (43).

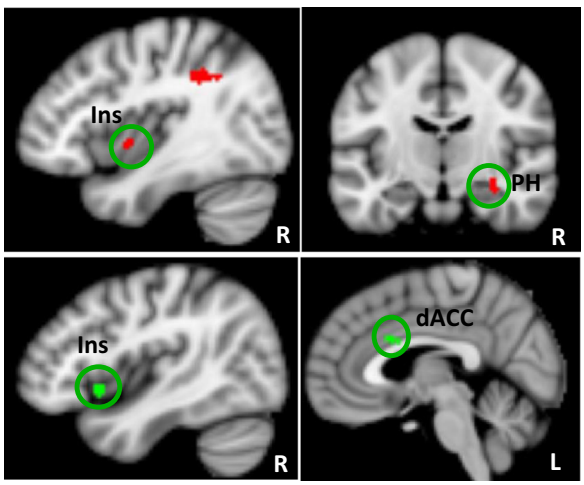

Figure 2. Red (top row) indicates brain regions that showed significant functional connectivity decrease with the default mode network in the transcutaneous vagus nerve stimulation group as compared with sham transcutaneous vagus nerve stimulation. Green color (bottom row) indicates brain regions whose default mode network functional connectivity changes (posttreatment minus pretreatment) were positively associated with the corresponding Hamilton Depression Rating Scale score changes across all subjects. dACC, dorsal anterior cingulate cortex; Ins, insula; L, left; PH, parahippocampus; R, right.

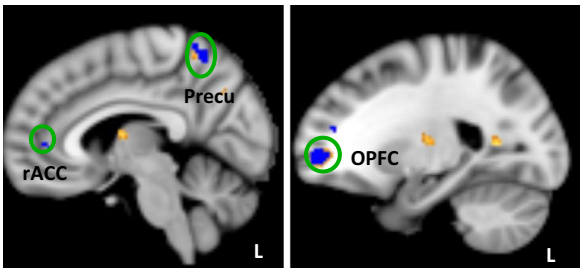

Figure 3. Yellow indicates brain regions that showed significant functional connectivity increase with default mode network in the transcutaneous vagus nerve stimulation group as compared with sham transcutaneous vagus nerve stimulation. Blue indicates brain regions whose default mode network functional connectivity changes (posttreatment minus pretreatment) were negatively correlated with the corresponding Hamilton Depression Rating Scale score changes across all subjects. L, left; OPFC, orbital prefrontal cortex; Precu, precuneus; rACC, rostral anterior cingulate cortex.

MDD is a common and complex disease with unknown pathogenesis (21,44). The monoamine hypothesis proposes that low levels of brain monoamines, such as serotonin, noradrenaline, and dopamine, are responsible for the development of MDD (21,44). Recently, accumulating evidence has suggested that the cholinergic system is also involved in the pathophysiology of depressive disorder (44,45). Specifically, it is believed that MDD is derived from the interaction of multiple genetic and environmental factors, which further causes high susceptibility to stress. Both chronic stress and depression are associated with cholinergic dysfunctions and structural and functional alterations in brain areas including the hippocampus, prefrontal cortex, and amygdala, which account for development of the cognitive symptoms of depression, such as attention deficit, memory impairment, and negativity bias (44). Based on regional glucose metabolism in MDD patients, Mayberg (46) and Mayberg *et al.* (47) posed a limbic-cortical dysregulation model. Based on the model, MDD is associated with metabolism decreases in dorsal limbic (anterior and posterior cingulate) and neocortical regions (prefrontal, premotor, parietal cortex) and relative increases in ventral paralimbic areas (subgenual cingulate, anterior insula, hypothalamus, caudate). MDD remission requires the inhibition of overactive ventral areas and disinhibition of underactive dorsal regions.

Despite the clinical application of VNS/tVNS for MDD patients, the underlying mechanism is not fully understood (3,48,49). Hypotheses are based on the impact of anatomy and function/neurotransmitter changes evoked by VNS/tVNS in mood control (50). The vagus nerve is a mixed nerve composed of about 80% afferent fibers. It is speculated that the antidepressant effects of VNS are partially attributed to the projection of afferent fibers to the nucleus tractus solitarius, which is further connected directly and indirectly with brain structures including the reticular formation, amygdala, hypothalamus, insula, thalamus, orbitofrontal cortex, and other limbic regions responsible for mood and anxiety regulation (51–54). Interestingly, some of these brain regions are also believed to be involved in the pathogenesis and remission of MDD (44–47), which builds a basis for treatment with VNS. In addition, both clinical and animal studies indicate that VNS can produce changes of neurotransmitters implicated in the pathogenesis of MDD (55), including serotonin (56), norepinephrine (57), gamma-aminobutyric acid, and glutamate (58).

The DMN is a network of brain regions that are active when the individual is not focused on the outside world and the brain is at wakeful rest. Previous studies have suggested that the DMN is associated with the self-referential system, affective cognition, and emotion regulation (25,26,29). MDD patients are characterized by an increase in self-focus and stress sensitivity (59). Thus, accumulating evidence indicates that the DMN plays an important role in neuropathology of MDD (30–36,60–62).

Recently, brain imaging tools have been applied to investigate the fMRI signal change evoked by tVNS (63–65). In an early study, Kraus *et al.* (63) found that robust tVNS can induce fMRI signal decreases in limbic brain areas, including the amygdala, hippocampus, parahippocampal gyrus, and middle and superior temporal gyrus, as well as an fMRI signal increase in the insula, precentral gyrus, and thalamus. Psychometric assessment revealed a significant improvement in well-being after tVNS. The control intervention (earlobe stimulation) did not show similar fMRI or psychometric effects. In a follow-up study (65), the authors also studied the anterior wall and posterior wall auditory canal separately. The results showed that stimulation of the anterior wall evoked significant limbic deactivation at the parahippocampal gyrus and the posterior cingulate cortex, as well as other regions including the thalamus, locus coeruleus, and solitary tract.

Consistent with the above brain imaging studies (63–65) that focused on the immediate brain response during tVNS, we found that after 1 month of tVNS treatment, the FC between the DMN and right anterior insula and parahippocampus significantly decreased.

Anterior insula belong to a salience network, which is involved in detecting and orienting to both external and internal salient stimuli and events (66,67). Investigators believe that the anterior insula is critical in maintaining and updating representations of current and predictive salience (66,68). Previous studies have suggested that the right anterior insula is involved in responses to salient stimuli via switching between the DMN-related self-referential network and executive network related to goal directed activities (66,69).

The parahippocampus is a key region in the limbic system. Sheline *et al.* (70) showed that depressed subjects looking at negative pictures elicited a significantly greater increase in activity in the parahippocampus and hippocampus than control subjects. Dillon *et al.* (71) found that control subjects, not MDD patients, showed a stronger encoding response to reward tokens in the parahippocampus and the dopaminergic midbrain. They hypothesized that the weaker memory for positive material in MDD patients reflects blunted encoding responses in the dopaminergic midbrain and medial temporal lobes in MDD patients.

We also found that after tVNS treatment, the FC between the DMN and left precuneus and the OPFC increased compared with stVNS. Furthermore, the DMN FC increase in these regions was also associated with the depression severity of the patients.

In a previous study, Li *et al.* (61) studied DMN resting state FC difference between MDD patients and healthy control

subjects, as well as the treatment effects of an antidepressant drug. They found that the FC between the posterior default mode subnetwork (similar to the DMN pattern derived from ICA in our study) and posterior cingulate cortex were normalized (decreased) after antidepressant treatment. In our study, we found that the FC between the DMN and precuneus increased after tVNS stimulation and the increase in FC was also associated with the depression severity reduction. This inconsistency may be due to different treatment modalities (61,72).

In this study, we found that the increase of DMN FC with the rostral ACC and mPFC is associated with a reduction in depression severity. This is consistent with results from a recent study on the treatment effect of transcranial magnetic stimulation in depression (34). These results also provide direct support to the hypothesis (18,47,73) that suggests that the rostral anterior cingulate cortex/mPFC plays an important role in MDD treatment.

We found the DMN and OPFC FC increased after tVNS treatment. This increase was associated with depression severity reduction. The OPFC is connected to neuroanatomical structures directly involved in emotional and executive processing, such as the hippocampal formation, amygdala, ventral striatum, anterior cingulate, hypothalamus, and medial temporal areas (74–76). Interestingly, we observed significant FC changes between the DMN and brain regions such as the parahippocampus, ACC, and medial temporal areas. These results suggest that the modulation of tVNS is not targeted to one particular region but rather can influence brain region networks associated with emotion/affect regulation. This result is consistent with the brain imaging studies during VNS/tVNS that found activity changes in multiple brain regions (55,63–65). Particularly, our study demonstrated that after longitudinal treatment, the FC between the DMN and some key regions in VNS pathways and mood regulation were significantly modified and some of the FC changes were associated with depression symptom relief.

There are several limitations in this study. First, this is not a randomized clinical trial. We used this strategy mainly due to ethical concerns. As the first study to use tVNS alone on patients suffering from mild and moderate depression, we thought it would be wise to first test the effectiveness of tVNS. After demonstrating that tVNS could significantly reduce patients' symptoms, we recruited a second cohort of patients to test if the treatment effect of tVNS was greater than that of the sham tVNS. Since the baseline characteristics were similar in the two cohorts of patients and the study was completed within 1 year, we do not expect the design will influence the validity of the study. Nevertheless, a randomized clinical trial is needed in the future.

Second, treatments in the study were self-administered by the MDD patients, and thus patient compliance may have influenced the observed results. To enhance compliance, all patients were required to complete daily entries in a diary that was checked during assessments. Nevertheless, this self-administration method provides direct evidence toward the feasibility of widespread application of the method used within the study, which could significantly reduce treatment expenses. Third, the treatment was only 4 weeks in duration; hence, the results obtained only represent short-term to mid-term effects. Further study is needed to evaluate the long-term effects of this treatment option. Also, objective control of the tVNS application (e.g., recording of the quantity or load of applied impulses) was not included, which prevented us from calculating dose-response relationships. Finally, the sample size was relatively small, and further study with a larger sample size is needed to replicate our study findings.

In summary, we found that tVNS can significantly reduce the severity of depression in patients. After tVNS, DMN FC showed significant changes in brain regions involved in emotional modulation. The FC changes were also associated with depression severity changes in MDD patients. Our findings provide a framework of the brain network to further understand the mechanism of tVNS treatment in MDD patients.

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http://www.chictr.org/cn/:Ear's vagal nerve stimulation for depression; http://www.chictr.org/cn/proj/show.aspx?proj=204; Chinese Clinical Trial Registry ChiCTR-TRC-11001201.

ARTICLE INFORMATION

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Transcutaneous Vagus Nerve Stimulation Modulates Default Mode Network in Major Depressive Disorder

Supplemental Information

Table S1. Baseline characteristics (mean ± SD) and comparison between two groups.

Items	**tVNS Group**	**stVNS Group**	***P* value**
n (male)	18 (5)	16 (5)	0.824
Age (y)	39.17 ± 14.85	42.56 ± 10.64	0.446
HAMD Score	28.50 ± 6.42	28.56 ± 4.79	0.974
HAMA Score	19.61 ± 5.88	16.88 ± 4.19	0.126
SAS Score	56.56 ± 8.69	58.50 ± 10.71	0.568
SDS Score	66.33 ± 11.11	66.94 ± 9.28	0.864

HAMD, Hamilton Depression Scale; HAMA, Hamilton Anxiety Scale; SAS, Self-Rating Anxiety Scale; SDS, Self-Rating Depression Scale; tVNS, transcutaneous vagus nerve stimulation; stVNS, sham tVNS.

Table S2. Brain regions showed significant functional connectivity post- and pre-treatment changes between the tVNS and stVNS groups

Contrast	Brain Region	Voxel Size	Peak Z Value	Peak Coordinate		
				X	Y	Z
tVNS > stVNS	left orbital prefrontal cortex	164	3.86	-30	58	-2
	right superior frontal gyrus	52	3.21	24	12	52
	right dorsal lateral prefrontal cortex	21	2.99	50	18	36
	left temporoparietal junction	413	3.84	-60	-62	30
	left fusiform gyrus	109	3.62	-56	-16	-24
	right fusiform gyrus	48	4.19	46	-40	-20
	right middle temporal gyrus	26	3.38	44	-56	-4
	right parahippocampus	63	3.76	16	-22	-18
	left precuneus	85	3.2	-2	-44	56
	left thalamus	182	4.21	-18	-14	12
	right thalamus	52	3.33	4	-4	10
stVNS > tVNS	left medial prefrontal cortex	39	3.15	-8	2	52
	right operculum	77	3.93	58	12	-4
	left operculum	64	4.54	-62	4	0
	left superior temporal gyrus	27	3.22	-54	12	-12
	right insula	22	3.14	38	0	-2
	right parahippocampus / hippocampus	31	3.31	32	-12	-10
	right inferior parietal lobule	93	3.81	38	-38	36
	left postcentral gyrus	78	3.87	-34	-44	62
	left lateral occipitotemporal gyrus	23	3.21	-42	-50	-18
	right middle occipital gyrus	55	3.37	30	-100	2
	left middle occipital gyrus	47	3.28	-26	-100	12
	left cerebellum	34	3.68	-10	-38	-36

Table S3. Brain regions showed significant association between the post- and pre-treatment HAMD score changes and corresponding functional connectivity changes across all treatment groups.

Association	Brain Region	Voxel Size	Peak Z Value	Peak Coordinate		
				X	Y	Z
Positive	left dorsal lateral prefrontal cortex	52	3.11	-46	10	40
	left orbital prefrontal cortex	162	3.57	-18	44	-16
	right operculum / anterior insula	55	3.29	54	10	-4
	right inferior temporal gyrus	22	3.18	36	-18	-36
	right dorsal anterior cingulate cortex	34	3.02	2	16	28
	right inferior parietal lobe	20	3.21	50	-42	52
	left middle occipital gyrus	21	3	-26	-100	14
	right cuneus	131	3.39	18	-84	36
	right pons	25	3.56	14	-26	-36
Negative	left dorsal lateral prefrontal cortex	25	3.23	-28	46	18
	left medial prefrontal gyrus	462	4.54	-4	62	4
	right dorsal lateral prefrontal cortex	28	3.06	30	48	8
	right orbital prefrontal cortex	54	3.31	42	46	-2
	right rostral anterior cingulate cortex	42	3.16	4	42	2
	left fusiform gyrus	175	3.59	-56	-12	-24
	left operculum	40	3.61	-64	-24	16
	left temporoparietal junction	27	3.02	-58	-66	30
	right fusiform gyrus	44	3.08	48	-2	-28
	right middle temporal gyrus	54	3.31	60	-16	-18
	right fusiform gyrus	33	3.2	48	-40	-20
	left operculum	29	3.55	-46	14	4
	right parahippocampus	25	3.04	22	-20	-22
	right precuneus	245	3.78	10	-54	58
	left insula	50	3.42	-42	-20	-4

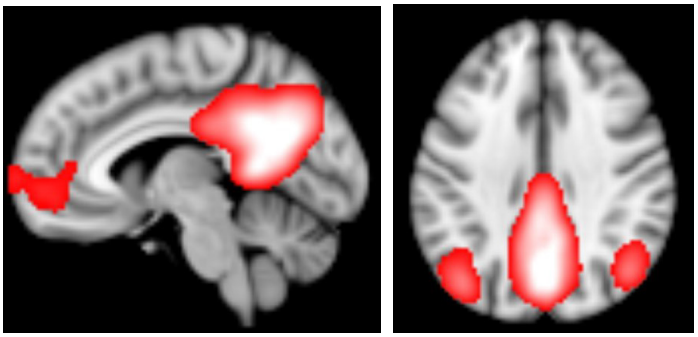

Figure S1. Default mode network derived from independent component analysis. Brain regions observed in the network include the medial prefrontal cortex, precuneus, posterior cingulate cortex and bilateral parietal cortex.