A single-center randomized, double-blind controlled trial of Transcutaneous Vagus Nerve stimulation (ta-VNS) for improvement of upper extremity motor function in stroke patients

Title	A single-center randomized, double-blind controlled trial of Transcutaneous Vagus Nerve stimulation (taVNS) for improvement of upper extremity motor function in stroke patients
Application	Department of Rehabilitation, Zhujiang Hospital, Southern Medical University
Purpose	Primary objectives: To investigate the facilitative effect of transcutaneous auricular vagus nerve stimulation with upper limb motor rehabilitation training on the improvement of upper limb motor function in stroke. Secondary objective: To examine the necessity of pairing with motor function training for transcutaneous auricular vagus nerve stimulation to improve upper limb dysfunction in stroke.
Research hypothesis	1) The test group will show significantly greater improvement in upper limb motor function in stroke patients compared to the control group. 2) Upper limb motor rehabilitation training paired with transcutaneous auricular vagus nerve stimulation will be significantly more effective in improving upper limb motor function in stroke patients compared with the unpaired test group.
Design	Exploratory single-center, double-blind, randomized controlled trial
Sample size	Sample size was determined: $n = 2*[(Z\alpha+Z\beta)\sigma/d]2$ (sample size estimated using the statistical effect force analysis software G*Power3.1). The sample size for this study was estimated as 40 study patients per group based on the effect size (effefect size, Cohend's d = 0.632) and $\alpha = 0.05$, $\beta = 0.80$ from the trial by Dawson et al. (2021). The sample size was adjusted to 50 patients per group based on the clinical sample dropout rate (20%). The final study population was 150 patients with ischemic stroke. Of these, 50 received upper extremity motor rehabilitation paired with transcutaneous auricular vagal stimulation, 50 received upper extremity motor rehabilitation with unpaired transcutaneous auricular vagal stimulation, and 50 received upper extremity motor rehabilitation paired with sham-stimulation (control group).

Eligibility	Inclusion Criteria: - Patients diagnosed with stroke according to a clinically qualified physician with reference to the Chinese Stroke Prevention and Control Guideline (2021) - Patients in the acute/recovery phase (after 2 weeks of onset) with stable signs - No previous neuropsychiatric-related diseases - No significant impairment of cognitive function and able to cooperate in completing the corresponding rehabilitation training - With unilateral upper limb dysfunction - Male or female, 18-80 years of age - Patients who have not received various neuromodulation rehabilitation treatments - No contraindications to taVNS - Subjects voluntarily cooperate with the study and agree to sign an informed consent Exclusion Criteria: - Patients have other mental health disorders (dementia, Parkinson's disease, depression, schizophrenia, bipolar disorder, etc.) - Patients have uncontrolled epilepsy, i.e., having had a seizure within 4 weeks prior to enrollment - Patients have cardiac arrhythmias or other abnormalities - Patients have a history of respiratory disease or disorder, including pneumonia, dyspnea and asthma - The presence of gastrointestinal disorders such as diarrhea and vomiting that make it difficult for the patient to cooperate - Patients have a history of vasovagal syncope - Patients are under treatment with other neurostimulation/modulation - The presence of severe spasticity, other serious injuries to the upper extremities, or other medical conditions - Patients have difficulty in understanding and communication, so they can’t complete the test - Women who are pregnant or breastfeeding
Major interventions	1) Paired taVNS: -Intervention: Upper extremity motor rehabilitation training will be conducted with paired taVNS. The stimulator will be placed on the left ear of the patient. While the patient performs the action training, the EMG signal feature caused by the action will trigger an electrical stimulation of the transcutaneous auricular vagus nerve. The amplitude of the electrical stimulation will be adjusted under patients’ pain threshold. The intervention will be performed once a day for 40-60 minutes for 14 days.

	2) Unpaired taVNS: -Intervention: Upper extremity motor rehabilitation training will be conducted with unpaired taVNS. The stimulator will be placed on the patient's left ear. Patients will receive 30 minutes of stimulation, with pulses of 500 ms occurring every 5-7 seconds. A total of 300 stimulations will be completed with pulses of 500 ms. After that motor training will be performed. The motor training will be identical to that of the paired group. The amplitude of the electrical stimulation will be adjusted under patients' pain threshold. The intervention will be conducted once a day, with 30 minutes of stimulation followed by 30 minutes of motor training each time for 14 days. 3) Sham taVNS: Upper limb motor rehabilitation training will be conducted with paired taVNS sham-stimulation. The stimulator will be placed on the patient's left ear. The amplitude of the electrical stimulation will be adjusted at 0 mA. The intervention will be conducted once a day for 40-60 minutes for 14 days.
Evaluating indicator	Primary outcome: The score of Fugl-Meyer Assessment of Upper Extremity (FMA-UE) will be tracked before and after the intervention in the paired taVNS, unpaired taVNS, and sham taVNS groups. Secondary outcome: Changes in ADL (Modified Barthel Index), the Hong Kong version of the Functional Test for the Hemiplegic Upper Extremity (FTHUE-HK), Wolf Motor Function Test (WMFT), EEG, and EMG features will be tracked after the intervention among participants.
Statistical method	1. Primary endpoints: ANOVA, paired-sample t-test, and independent-sample t-test will be used. (1) For the main efficacy index FMA-UE scores, paired sample t-test will be performed before and after the intervention to compare whether there will be a significant change before and after the intervention. Since this statistical operation is based on multiple comparisons, there may be a bias of alpha inflation. Therefore, the statistical results will be corrected based on Bonferroni's principle. (2) The FMA-UE scores before and after the intervention will be summed and subtracted to obtain the FMA-UE change scores of the indicators before and after the intervention (i.e., the main intervention effect), and a one-way three-level (paired vs. unpaired vs. pseudo-stimulated) analysis of variance (ANOVA) will be performed on the main intervention effect of the three groups to obtain the variability of the main intervention effect of the three groups. (3) Independent samples t-tests will be conducted for the main intervention effects among groups. The main intervention effects of the two experimental groups will be obtained. And the main intervention effects of the paired experimental group and the unpaired experimental group will be compared by independent sample t-test.

	2. Secondary endpoints: ANOVA, paired samples t-test, independent samples t-test (1) Paired-samples t-tests will be performed before and after the intervention for WMFT, Brunnstrom scale, Modified Barthel Index, FTHUE-HK, and EMG characteristics to compare whether there will be significant changes before and after the intervention. Since this statistical manipulation is based on multiple comparisons and there would be a bias for alpha inflation, and the statistical results will be corrected based on Bonferroni's principle. (2) The WMFT, Brunnstrom scale, Modified Barthel Index, FTHUE-HK, and EMG characteristics before and after the intervention were summed and subtracted. To obtain the WMFT, Brunnstrom assessment, ADL, FTHUE-HK, and changes in EMG characteristics of the indicators before and after the intervention (i.e., secondary intervention effects), and the secondary intervention effects of the three groups were subjected to a one-way three-level (paired vs. unpaired vs. pseudo-stimulation) multiple test analysis of variance (Multivariate ANOVA) to obtain the variability of the secondary intervention effects in the three groups. 3) The WMFT, Brunnstrom scale, Modified Barthel Index, FTHUE-HK, and change in EMG characteristics of the paired and unpaired test groups will be tested by independent samples t-tests to obtain the difference of secondary intervention effects between paired and unpaired groups. To compensate the alpha inflation bias, the statistical results will be corrected based on Bonferroni's principle.

#**Table 1** Schematic timeline of the study

TIMEPOINT	Enrollment	Allocation	Post-Allocation				Close-out
	ts	t0	t1	t2	t3	t4	t5
Enrolment	×						
Eligibility screen	×						
Informed consent	×						
Demographic information	×						
Medical history	×						
Vital signs	×		×	×	×	×	×
Randomization		×					
Allocation		×					
Interventions							
Paired ta-VNS				×			

ta-VNS				×			
Sham ta-VNS				×			
Assessments							
FMA-UE			×		×	×	×
WMFT			×		×	×	×
Brunnstrom			×		×	×	×
Modified Barthel Index			×		×	×	×
FTHUE-HK			×		×	×	×
Adverse events	×	×	×	×	×	×	×

1) Demographic information includes the patients date of birth, sex and race;
2) Medical history includes stroke and other clinically significant past and present medical history and pre-existing/combined medications;
3) Vital signs include blood pressure, pulse rate, respiration and body temperature;

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