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Transcutaneous auricular vagus nerve stimulation for minimally conscious state: an exploratory randomized controlled trial with TMS-EEG assessment

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Abstract

Background: Non-invasive interventions that can be delivered repeatedly at the bedside are needed to support rehabilitation in people with minimally conscious state (MCS). We examined behavioral responses to four weeks of transcutaneous auricular vagus nerve stimulation (taVNS) and associated cortical responses assessed with transcranial magnetic stimulation-electroencephalography (TMS-EEG).

Methods: In this single-center, exploratory randomized trial, 40 participants with MCS were allocated 1:1 to taVNS or transcutaneous non-vagal nerve stimulation (tnVNS), with blinded intervention delivery and outcome assessment. Stimulation was delivered for 30 min, three times daily, 5 days per week for four weeks. The primary behavioral outcome was change in Coma Recovery Scale-Revised (CRS-R) total score. TMS-EEG outcomes included significant current density (SCD) and significant current scattering (SCS). Group-by-time effects were assessed using repeated-measures analysis of variance; correlations with behavioral changes were exploratory.

Results: All 40 participants completed the intervention and assessments. Mean CRS-R change was 3.55 ± 1.88 points with taVNS and 0.15 ± 1.18 with tnVNS (between-group difference 3.40 points, 95% confidence interval 2.40–4.40; $p < 0.001$). Group-by-time interactions were observed for SCD ($F(1,38) = 10.18$, $p = 0.0028$) and all three SCS comparisons ($p = 0.0007$–0.0268). Exploratory correlations linked SCD and SCS changes with CRS-R improvement. Late scalp-level TMS-evoked responses increased within the taVNS group. No stimulation-related adverse events were reported.

Conclusions: Four weeks of taVNS was associated with greater behavioral improvement than tnVNS and with changes in TMS-evoked cortical responses. These findings support further evaluation of taVNS in neurorehabilitation and of TMS-EEG measures as candidate treatment-response markers; they do not establish predictive or clinical decision-making utility.

Trial registration: International Traditional Medicine Clinical Trial Registry, ITMCTR2024000734.

Background

Disorders of consciousness (DOC), including unresponsive wakefulness syndrome and minimally conscious state (MCS), are severe neurological conditions after acquired brain injury and remain among the most challenging disorders to treat [1–4]. MCS is clinically important because participants retain reproducible but fluctuating signs of conscious processing, indicating that residual thalamocortical and corticocortical networks may still be accessible to therapeutic modulation [1, 3]. However, effective interventions for promoting recovery of consciousness remain limited. Current pharmacological and neuromodulatory approaches have shown encouraging effects in selected participants, but the overall evidence remains insufficient to support broadly applicable treatment algorithms [3, 4]. Therefore, there is a pressing clinical need for safe, repeatable, and bedside-deliverable interventions that can promote behavioral responsiveness while allowing objective monitoring of treatment response.

Transcutaneous auricular vagus nerve stimulation (taVNS) has emerged as a promising non-invasive neurotherapeutic candidate for consciousness recovery. Compared with implanted vagus nerve stimulation, taVNS can be delivered repeatedly at the bedside, avoids surgical implantation, and may provide a practical therapeutic strategy for participants with severe neurological impairment. Previous studies of implanted VNS and taVNS in DOC have reported behavioral and neurophysiological improvements in case reports, cohort studies, and randomized trials [5–9]. The mechanistic rationale is that auricular vagal afferents provide a peripheral entry point to the nucleus tractus solitarius and downstream neuromodulatory systems, including locus-coeruleus noradrenergic, basal-forebrain cholinergic, and thalamocortical arousal pathways [10–12]. These systems are closely related to cortical arousal, excitation-inhibition balance, and large-scale network integration, all of which are critical for conscious processing [13, 14]. Nevertheless, treatment-related cortical network mechanisms of taVNS in MCS remain incompletely understood.

A major barrier to developing taVNS as a neurotherapeutic strategy for MCS is the lack of objective biomarkers that can quantify whether and how the injured brain responds to treatment. Behavioral assessment with the Coma Recovery Scale-Revised (CRS-R) remains essential for diagnosing and monitoring participants with DOC [15]. However, behavioral output in MCS is often limited, fluctuating, and vulnerable to confounding by arousal state, motor impairment, and examiner variability. Recent work on cognitive motor dissociation further emphasizes that clinically observable behavior may underestimate residual cortical function in participants with DOC [16]. Accordingly, current expert recommendations highlight the importance of integrating objective neurophysiological tools into the assessment, prognostication, and treatment monitoring of DOC [17]. A candidate response-monitoring measure should first demonstrate a reproducible association with treatment-related change before it can be evaluated for prospective participant stratification or parameter selection.

Concurrent transcranial magnetic stimulation and electroencephalography (TMS-EEG) provides such a perturbational approach. By delivering a controlled cortical perturbation and recording the resulting electrophysiological response with millisecond temporal resolution, TMS-EEG can assess cortical excitability, effective network engagement, and the large-scale propagation of evoked activity [18, 19]. TMS-EEG has been used to distinguish levels of consciousness and to reveal impaired cortical effective connectivity and complexity in DOC [20–23]. Recent TMS-EEG evidence further indicates that disrupted TMS-evoked information flow is closely associated with impaired consciousness [19]. In the context of taVNS, TMS-EEG may therefore serve not only as a mechanistic tool but also as a candidate measure of treatment-related cortical responses. A previous randomized crossover study in MCS combined rTMS with TMS-EEG complexity assessment [24]; the present study examines complementary source-response magnitude and spatial-spread measures during an auricular stimulation trial.

In this randomized, transcutaneous non-vagal nerve stimulation (tnVNS)-controlled, blinded trial, we investigated whether a four-week taVNS regimen improves behavioral responsiveness in participants with MCS and whether treatment response is accompanied by changes in perturbation-evoked cortical network dynamics. Behavioral responsiveness was assessed using CRS-R, and TMS-EEG was used to

quantify scalp-level TMS-evoked potentials, global mean field power, source-level significant current density, and significant current scattering. We hypothesized that taVNS, compared with tnVNS, would improve CRS-R scores and enhance TMS-EEG-derived measures of cortical excitability and large-scale frontoparietal/interhemispheric propagation. By linking behavioral improvement to objective perturbational biomarkers, this study aimed to evaluate taVNS as a bedside-deliverable non-invasive therapeutic strategy for MCS and to explore TMS-EEG markers for exploratory treatment-response assessment.

Methods

Study design and ethics

This was a single-center, exploratory, parallel-group randomized trial comparing taVNS with tnVNS in participants with MCS. Forty participants were allocated 1:1 to four weeks of treatment. Intervention delivery and outcome assessment were blinded using a specially designed multi-contact auricular clip system, as detailed below.

The study was conducted at Aviation General Hospital between October 2024 and December 2025. The protocol was approved by the Ethics Committee of the Institute of Acupuncture and Moxibustion, China Academy of Chinese Medical Sciences (S2024-01-31-13). Written informed consent was obtained from the legal representative of each participant before enrolment. The trial was registered at the International Traditional Medicine Clinical Trial Registry under the identifier ITMCTR2024000734. Participant enrolment, allocation, follow-up, and analysis are summarized in the CONSORT flow diagram.

Participants

Participants were eligible if they met the following criteria: (1) diagnosis of MCS based on established diagnostic criteria [1, 15] and repeated assessment with the Coma Recovery Scale-Revised (CRS-R); (2) acquired brain injury caused by traumatic brain injury, cerebral hemorrhage, or anoxic-ischemic encephalopathy; (3) age between 18 and 80 years; (4) disease duration greater than 28 days and less than 2 years after brain injury; (5) stable vital signs and clinical condition sufficient to complete the four-week intervention and TMS-EEG recordings; and (6) written informed consent provided by the participant's legal representative.

Exclusion criteria were as follows: (1) uncontrolled epilepsy or recent frequent seizures; (2) contraindications to TMS, including intracranial metallic implants, implanted electronic devices, or unstable skull defects; (3) localized skin lesions, infection, ulceration, bleeding, or severe irritation at the auricular stimulation sites; (4) bradycardia, clinically significant arrhythmia, or unstable autonomic or cardiovascular status; (5) deep sedation induced by general anesthesia or sedative medication; (6) clinically critical conditions, including acute myocardial infarction, pulmonary embolism, sepsis, severe pulmonary infection, intracranial infection, or brain herniation; (7) medications or concurrent

interventions expected to substantially affect cortical excitability during the study period; and (8) inability to complete usable TMS-EEG recordings because of excessive artifacts or clinical instability.

Randomization and blinding

Participants were randomized to the taVNS or tnVNS group using a computer-generated random sequence. Allocation was concealed using opaque, sealed, sequentially numbered envelopes prepared by personnel who were not involved in participant recruitment, intervention delivery, CRS-R assessment, TMS-EEG preprocessing, or statistical analysis.

A specially designed multi-contact auricular clip system was used to support treatment blinding. The taVNS and tnVNS clips were identical in external appearance, were connected to the same stimulation device, and were placed at the same predefined auricular position in all participants. According to the allocation code, electrical output was selectively routed to clip contacts overlying either vagus-innervated auricular regions (taVNS) or non-vagal regions at the auricular margin (tnVNS), without revealing group assignment to the treating personnel. Therefore, the intervention procedures, devices, stimulation schedules, and visible clip placement procedures were matched between groups.

Group allocation was concealed from treating personnel, CRS-R assessors, TMS-EEG preprocessing investigators, and statistical analysts. The allocation code was held by an independent researcher and was not disclosed until completion of the primary behavioral and TMS-EEG analyses. Participants were not informed of their allocation; because participants had MCS, their perceived stimulation condition could not be reliably assessed.

Interventions

taVNS and tnVNS were delivered using identical-appearing auricular electrical stimulators (Hwato SDZ-IIB, Suzhou Medical Supplies Factory, Suzhou, China). Before each stimulation session, the auricular skin was cleaned with alcohol to reduce impedance and ensure stable contact.

In the taVNS group, bilateral auricular clips were applied, and electrical output was routed to clip contacts overlying vagus-innervated auricular regions involving the conchal area. The sites were described using the traditional auricular terms Heart and Kidney. Electrical stimulation was delivered at 20 Hz and 1.0-1.5 mA. Each session lasted 30 min, and participants received three sessions per day, 5 days per week, for four consecutive weeks.

In the tnVNS group, the same device, stimulation frequency, intensity range, session duration, daily frequency, and treatment schedule were used. However, electrical output was routed to clip contacts overlying the auricular margin, a site outside the main distribution of the auricular branch of the vagus nerve. This control condition was intended to match procedural exposure and non-specific sensory stimulation while minimizing auricular vagal afferent engagement.

Outcomes

The primary behavioral outcome was the pre-to-post intervention change in CRS-R total score. CRS-R assessments were performed before the intervention and after four weeks of treatment under clinically stable conditions and, whenever possible, at comparable times of day to reduce variability related to fluctuating arousal.

Secondary mechanistic outcomes were TMS-EEG-derived measures of perturbation-evoked cortical network responses, including scalp-level TMS-evoked potentials (TEPs), global mean field power (GMFP), source-level significant current density (SCD), and significant current scattering (SCS). Exploratory analyses examined associations between changes in CRS-R scores and changes in TMS-EEG measures.

Safety and tolerability

Safety and tolerability were monitored throughout the study period. All adverse events were recorded regardless of their presumed relationship to the intervention. Case-report forms documented the timing, symptoms and signs, severity, duration, relevant examinations, treatment, outcome, and investigator-assessed causality. Particular attention was paid to auricular pain, erythema, ulceration, bleeding, dizziness, bradycardia, seizures, autonomic instability, infection, and clinical deterioration. Serious adverse events were immediately reported to the principal investigator and managed according to the study protocol.

Safety outcomes included the occurrence of stimulation-related adverse events, serious adverse events, and discontinuation due to adverse events. The number and type of adverse events were summarized descriptively for each group.

TMS-EEG acquisition

TMS procedures followed current safety recommendations [25]. TMS-EEG was recorded using 64 MR-compatible electrodes and BrainAmp amplifiers (Brain Products, Germany) at 2500 Hz. TMS was delivered with a Magstim Rapid2 stimulator connected to a 70-mm air-cooled figure-of-eight coil (Magstim, UK). Electrode impedances were kept below 5 kΩ. White noise through earplugs and a thin foam spacer between coil and scalp were used to reduce auditory and somatosensory confounds. Resting motor threshold (RMT) was measured, and stimulation intensity was set to 90% of RMT to reduce electromyographic contamination. Single-pulse TMS was delivered over the left dorsolateral prefrontal cortex (F3 position of the 10–20 system), with the coil tangential to the scalp and angled approximately 45 degrees relative to the midline. Each session included 150 trials with random inter-trial intervals of 2.1–3.1 seconds.

TMS-EEG preprocessing

TMS-EEG preprocessing was performed in MATLAB using EEGLAB and the TESA plugin [26, 27]. Continuous data were epoched from − 1000 to + 1000 ms around each TMS pulse and baseline-corrected using the − 1000 to 0 ms pre-stimulus period. Trials and channels with excessive noise, muscle activity, eye movement artifacts, or other non-physiological artifacts were visually inspected and rejected. Data were then downsampled to 1000 Hz and bandpass filtered from 1 to 80 Hz. Independent component analysis (ICA) was used to remove residual TMS-related artifacts as well as ocular and muscle components. The cleaned data were re-referenced to the common average.

Scalp-level TMS-EEG analysis

Scalp-level TEPs were visualized using butterfly plots. GMFP was calculated as the across-electrode standard deviation of voltage at each time point and was used to quantify the global strength of TMS-evoked scalp responses. TEP morphology, GMFP time courses, and topographical distributions were compared before and after intervention within each group.

Source-level analysis and TMS-EEG network measures

Source analysis was performed in Brainstorm [28]. A boundary-element method (BEM) head model was constructed with OpenMEEG [29] using a template anatomy. Source estimates were obtained with minimum-norm imaging and standardized using standardized low-resolution brain electromagnetic tomography (sLORETA) principles [30]. Cortical parcels were defined using the Desikan-Killiany atlas [31]. Significant source activity was identified by comparison with baseline (-300 to 0 ms) using parametric t-tests with Bonferroni correction. SCD quantified the summed absolute amplitude of significant cortical currents within 0-500 ms after TMS. SCS quantified the spatial dispersion of significant sources from the left frontal stimulation target to left parietal (F3-P3), right parietal (F3-P4), and right frontal (F3-F4) regions.

Sample size rationale

This trial was designed as an exploratory mechanistic study of taVNS in participants with MCS. The sample size was determined based on feasibility and previous clinical studies of taVNS in disorders of consciousness rather than a formal efficacy-powered calculation. The findings should therefore be interpreted as preliminary and hypothesis-generating, requiring validation in larger multicenter trials.

Statistical analysis

Statistical analyses were performed using MATLAB R2022b. Continuous variables are presented as mean ± standard deviation. Within-group pre-to-post differences were tested using paired-sample t-tests, and t values, p values and Cohen's d were calculated. Between-group comparisons were performed using independent-sample t-tests or Welch-corrected tests when variances were unequal. Repeated-measures analysis of variance (ANOVA) was used to examine the main effects of group and time and the group × time interaction. When appropriate, F values, p values and partial η^2 were reported.

Channel-wise TEP differences and time-wise GMFP differences were evaluated using Wilcoxon signed-rank tests with Benjamini-Hochberg false-discovery-rate (FDR) correction. To reduce false-positive findings at isolated time points, significant GMFP effects were interpreted only when they occurred over consecutive time windows.

Associations between treatment-related changes in CRS-R scores and TMS-EEG measures were assessed using Spearman correlation analysis. To test statistical significance without assuming a specific distribution, permutation testing was performed by randomly shuffling CRS-R change labels 1000 times and recalculating the correlation coefficient to generate an empirical null distribution. Two-sided empirical p values were calculated as the proportion of permuted correlation coefficients with absolute values greater than or equal to the observed correlation coefficient. Statistical significance was set at $p < 0.05$.

Use of generative AI in manuscript preparation

ChatGPT (OpenAI) was used to assist with language revision, manuscript organization, and preparation of submission materials. The authors are responsible for the final scientific content.

Results

Participant flow and baseline characteristics

Between October 2024 and December 2025, 60 participants with MCS were assessed for eligibility; 20 were excluded (10 did not meet the inclusion criteria and 10 declined to participate), and 40 participants were randomized to the tnVNS (n = 20) or taVNS (n = 20) group. All randomized participants completed the four-week intervention and all assessments, with no loss to follow-up (Fig. 1A). Each group included 10 women and 10 men. Mean age was 46.85 ± 15.32 years in the tnVNS group and 42.15 ± 13.55 years in the taVNS group. Etiologies were balanced between groups, with traumatic brain injury (n = 8), cerebral hemorrhage (n = 8), and anoxic-ischemic encephalopathy (n = 4) in each group. Mean disease duration was 8.35 ± 2.03 months in the tnVNS group and 9.20 ± 3.14 months in the taVNS group. There were no significant baseline differences in age ($t(38) = 1.03$, $p = 0.311$), disease duration ($t(38) = -1.02$, $p = 0.316$), or baseline CRS-R score ($t(38) = 0.96$, $p = 0.343$). The distribution of MCS − and MCS+ subtypes was 14/6 in the tnVNS group and 19/1 in the taVNS group (Fisher exact test, $p = 0.091$). Individual-level baseline data are provided in Table 1.

Table 1
Individual-level baseline demographic and clinical characteristics.

Group	Sex	Age, years	Etiology	Duration, months	CRS-R	Diagnosis
tnVNS-P1	female	24	anoxic	9	10	MCS-
tnVNS-P2	male	54	hemorrhage	7	9	MCS-
tnVNS-P3	female	51	TBI	7	12	MCS+
tnVNS-P4	male	59	TBI	9	10	MCS-
tnVNS-P5	male	35	anoxic	8	12	MCS+
tnVNS-P6	female	54	hemorrhage	7	8	MCS-
tnVNS-P7	male	59	anoxic	12	7	MCS-
tnVNS-P8	female	39	hemorrhage	10	8	MCS-
tnVNS-P9	female	45	TBI	9	8	MCS-
tnVNS-P10	male	44	hemorrhage	7	10	MCS-
tnVNS-P11	male	80	TBI	11	15	MCS+
tnVNS-P12	female	33	TBI	5	12	MCS+
tnVNS-P13	female	42	anoxic	13	10	MCS-
tnVNS-P14	male	24	TBI	6	8	MCS-
tnVNS-P15	female	78	hemorrhage	6	8	MCS-
tnVNS-P16	male	41	TBI	8	12	MCS+
tnVNS-P17	female	53	hemorrhage	9	9	MCS-
tnVNS-P18	male	54	hemorrhage	9	10	MCS-
tnVNS-P19	female	39	TBI	8	10	MCS-

Abbreviations: CRS-R, Coma Recovery Scale-Revised; MCS, minimally conscious state; TBI, traumatic brain injury; taVNS, transcutaneous auricular vagus nerve stimulation; tnVNS, transcutaneous non-vagal nerve stimulation.

Group	Sex	Age, years	Etiology	Duration, months	CRS-R	Diagnosis
tnVNS-P20	male	29	hemorrhage	7	12	MCS+
taVNS-P1	male	54	TBI	8	11	MCS-
taVNS-P2	male	42	hemorrhage	5	9	MCS-
taVNS-P3	female	44	anoxic	6	9	MCS-
taVNS-P4	female	40	anoxic	11	9	MCS-
taVNS-P5	female	60	hemorrhage	8	10	MCS-
taVNS-P6	male	24	hemorrhage	5	10	MCS-
taVNS-P7	male	45	TBI	12	13	MCS+
taVNS-P8	female	70	hemorrhage	9	10	MCS-
taVNS-P9	male	32	TBI	16	9	MCS-
taVNS-P10	male	35	TBI	6	10	MCS-
taVNS-P11	female	26	hemorrhage	8	8	MCS-
taVNS-P12	female	61	TBI	8	9	MCS-
taVNS-P13	female	18	anoxic	11	9	MCS-
taVNS-P14	male	29	hemorrhage	6	8	MCS-
taVNS-P15	female	40	TBI	12	9	MCS-
taVNS-P16	male	33	hemorrhage	13	8	MCS-
taVNS-P17	female	52	TBI	9	11	MCS-
taVNS-P18	male	52	TBI	6	10	MCS-
taVNS-P19	female	50	hemorrhage	12	9	MCS-

Abbreviations: CRS-R, Coma Recovery Scale-Revised; MCS, minimally conscious state; TBI, traumatic brain injury; taVNS, transcutaneous auricular vagus nerve stimulation; tnVNS, transcutaneous non-vagal nerve stimulation.

Group	Sex	Age, years	Etiology	Duration, months	CRS-R	Diagnosis
taVNS-P20	male	36	anoxic	13	9	MCS-

Abbreviations: CRS-R, Coma Recovery Scale-Revised; MCS, minimally conscious state; TBI, traumatic brain injury; taVNS, transcutaneous auricular vagus nerve stimulation; tnVNS, transcutaneous non-vagal nerve stimulation.

taVNS improved behavioral responsiveness after four weeks

After the four-week intervention, CRS-R scores did not significantly change in the tnVNS group, increasing only from 10.00 ± 2.00 at baseline to 10.15 ± 1.79 after treatment ($t(19) = -0.57$, $p = 0.58$, Cohen's $d = 0.13$). In contrast, CRS-R scores significantly increased in the taVNS group, from 9.50 ± 1.19 at baseline to 13.05 ± 2.09 after treatment ($t(19) = -8.46$, $p < 0.001$, Cohen's $d = 1.89$) (Fig. 1B). Repeated-measures ANOVA showed a significant main effect of group ($F(1,38) = 5.48$, $p = 0.02$, partial $\eta^2 = 0.13$) and a significant group × time interaction ($F(1,38) = 46.98$, $p < 0.001$, partial $\eta^2 = 0.55$), indicating greater improvement in behavioral responsiveness after taVNS than after tnVNS. The between-group difference in ΔCRS-R was significant (taVNS: 3.55 ± 1.88; tnVNS: 0.15 ± 1.18; mean difference = 3.40 points, 95% CI 2.40–4.40; $t(38) = 6.85$, $p < 0.001$, Cohen's $d = 2.17$) (Fig. 1C), indicating that the taVNS group had significantly greater CRS-R improvement than the tnVNS group.

Scalp-level TMS-evoked responses

Within-group scalp-level TMS-EEG analyses characterized pre-to-post changes in each intervention arm. In the tnVNS group, TMS-evoked potential morphology showed no obvious change after intervention, and response amplitudes around 100 ms and 160 ms remained largely unchanged (Fig. 2). In contrast, the taVNS group showed enhanced TMS-evoked responses after treatment, particularly around the late response window near 160 ms. GMFP analysis showed significant increases after taVNS in the 20–30 ms, 90–100 ms, and 140–160 ms windows, whereas the tnVNS group showed minimal change. Topographical analysis further indicated that significant electrode-level changes after taVNS were distributed mainly over frontocentral and posterior regions.

Source-level response magnitude

Source reconstruction revealed no substantial intervention-related change in the tnVNS group, with significant activations remaining mainly in bilateral frontal and parieto-occipital regions. In contrast, after taVNS, a greater number of significantly activated cortical regions was observed, including left frontal, motor, right premotor, and parieto-occipital areas (Fig. 3). Source-level responses in the taVNS group were especially enhanced during the 100–200 ms post-stimulation window.

Quantitative SCD analysis supported these source-level observations. In the tnVNS group, SCD showed no significant change after intervention (11.03 ± 6.44 to 9.43 ± 4.88; t(19) = 1.31, p = 0.21, Cohen's d = -0.29). In contrast, SCD significantly increased after taVNS (13.70 ± 5.60 to 16.59 ± 5.61; t(19) = -4.1549, p = 0.0005, Cohen's d = 0.93). Repeated-measures ANOVA showed a significant group effect (F(1,38) = 8.91, p = 0.0049, partial η^2 = 0.19) and a significant group × time interaction (F(1,38) = 10.18, p = 0.0028, partial η^2 = 0.21). The change rate of SCD was positively correlated with the change rate of CRS-R score (r = 0.79; permutation p < 0.05), suggesting that increased perturbation-evoked cortical excitability was associated with behavioral improvement.

Spatial spread of source activity and exploratory behavioral associations

SCS was used to quantify the spatial propagation of significant TMS-evoked source activity from the left frontal stimulation target to distant cortical regions. The tnVNS group showed no significant SCS change along any pathway. In contrast, the taVNS group showed significant increases in all three predefined propagation pathways (Table 2; Fig. 4). Specifically, SCS increased along the ipsilateral frontoparietal F3-P3 pathway from 11.19 ± 6.66 to 15.72 ± 8.79 (t(19) = -3.39, p = 0.0031, Cohen's d = 0.76), along the cross-hemispheric frontoparietal F3-P4 pathway from 16.29 ± 9.96 to 21.37 ± 11.99 (t(19) = -3.85, p = 0.0011, Cohen's d = 0.86), and along the interhemispheric frontal F3-F4 pathway from 11.41 ± 6.74 to 14.90 ± 6.63 (t(19) = -3.27, p = 0.0040, Cohen's d = 0.73).

Table 2
Changes in significant current scattering and ANOVA results.

Variable	F3-P3	F3-P4	F3-F4
tnVNS group	13.17 ± 10.66 to 12.07 ± 9.61; t(19) = 1.48, p = 0.1559, Cohen's d = -0.33	14.41 ± 12.40 to 12.80 ± 9.20; t(19) = 1.17, p = 0.2572, Cohen's d = -0.26	12.38 ± 8.82 to 12.81 ± 8.60; t(19)=-0.54, p = 0.5945, Cohen's d = 0.12
taVNS group	11.19 ± 6.66 to 15.72 ± 8.79; t(19)=-3.39, p = 0.0031, Cohen's d = 0.76	16.29 ± 9.96 to 21.37 ± 11.99; t(19)=-3.85, p = 0.0011, Cohen's d = 0.86	11.41 ± 6.74 to 14.90 ± 6.63; t(19)=-3.27, p = 0.0040, Cohen's d = 0.73
Group effect	F(1,38) = 0.09, p = 0.77, partial η^2=0.0024	F(1,38) = 2.46, p = 0.13, partial η^2=0.06	F(1,38) = 0.06, p = 0.81, partial η^2=0.0015
Group × time	F(1,38) = 13.54, p = 0.0007, partial η^2=0.26	F(1,38) = 12.31, p = 0.0012, partial η^2=0.24	F(1,38) = 5.31, p = 0.0268, partial η^2=0.12

Abbreviations: ANOVA, analysis of variance; SCS, significant current scattering; taVNS, transcutaneous auricular vagus nerve stimulation; tnVNS, transcutaneous non-vagal nerve stimulation.

Repeated-measures ANOVA showed significant group × time interactions for F3-P3 ($F(1,38) = 13.54$, $p = 0.0007$, partial $\eta^2 = 0.26$), F3-P4 ($F(1,38) = 12.31$, $p = 0.0012$, partial $\eta^2 = 0.24$), and F3-F4 ($F(1,38) = 5.31$, $p = 0.0268$, partial $\eta^2 = 0.12$). SCS change rates were positively associated with CRS-R change rate for F3-P3 ($r = 0.77$), F3-P4 ($r = 0.70$), and F3-F4 ($r = 0.65$), indicating that stronger long-range cortical propagation was associated with greater behavioral recovery after taVNS.

Safety and tolerability

No stimulation-related adverse events or serious adverse events were reported in either group during the four-week intervention period. No participant discontinued the intervention because of an adverse event.

Discussion

In this exploratory randomized trial, the taVNS group showed greater improvement in CRS-R score than the tnVNS group over four weeks, with a between-group difference in change of 3.40 points (95% CI 2.40–4.40). Group-by-time effects for SCD and SCS provide evidence of accompanying changes in TMS-evoked source activity. Scalp-level analyses and correlations with behavioral change offer complementary exploratory observations. This combination of behavioral assessment and perturbational neurophysiology is relevant to neurorehabilitation, but it does not yet validate these measures for treatment selection or prediction.

The present findings are clinically relevant because MCS remains a therapeutic challenge with limited evidence-based interventions. Participants with MCS retain inconsistent but reproducible signs of conscious processing, implying that residual large-scale networks may remain modifiable. In this context, taVNS has several features that make it attractive as a therapeutic candidate for consciousness recovery: it is non-invasive, repeatable, relatively easy to deliver at the bedside, and does not require surgical implantation. Previous studies of implanted VNS and taVNS in DOC have reported encouraging behavioral and neurophysiological effects, but the evidence base remains limited and heterogeneous [3–9]. Our study extends this literature by showing that a structured four-week taVNS regimen was associated not only with improved CRS-R scores but also with objective changes in cortical network responsiveness. The four-week schedule is comparable to treatment windows used in previous DOC studies [6–9]. Its relevance here is the concurrent assessment of behavioral change and TMS-evoked responses, rather than novelty of treatment duration.

TMS-EEG was used as an exploratory response-monitoring tool rather than as a validated biomarker. It complements behavioral assessment by probing cortical responses to an external perturbation [18, 20]. SCD summarizes the magnitude of statistically significant source activity, while SCS summarizes its spatial spread; these operational measures should not be equated with isolated neuronal excitability or a direct measurement of anatomical connectivity. The correlations with CRS-R change are hypothesis-generating. Prospective prediction, repeatability, independent validation, and incremental value over behavioral assessment remain to be established.

SCS increased after taVNS for the F3–P3, F3–P4, and F3–F4 region pairs. This pattern is consistent with a wider distribution of TMS-evoked activity across frontal and parietal regions, which is relevant to models of distributed conscious processing [13, 14, 19, 20]. However, greater spatial spread is not synonymous with restored information integration, and SCS alone does not establish direction-specific communication. These measures should be interpreted alongside, rather than as substitutes for, validated complexity and effective-connectivity measures [21, 22, 24].

Ascending neuromodulatory pathways provide a plausible framework for auricular stimulation effects. Brainstem responses to transcutaneous vagus nerve stimulation and preclinical observations during vagus nerve stimulation suggest possible engagement of arousal-related systems [10–12]. However, evidence from implanted VNS cannot by itself establish identical pathway engagement by auricular stimulation. This study did not measure nucleus tractus solitarius, locus coeruleus, basal-forebrain, or thalamic activity, and therefore cannot identify a necessary or sufficient pathway, or establish that cortical changes mediated behavioral improvement.

The randomized comparator and externally matched multi-contact clip system support the interpretation of treatment-related differences. The combination of clinical assessment and TMS-EEG also permits comparison of behavioral and neurophysiological changes within the same trial. These strengths do not remove the need for precise intervention reporting, transparent preprocessing, and independent replication.

Several limitations warrant consideration. This single-center exploratory trial was small, included heterogeneous etiologies, and had a numerical imbalance in MCS−/MCS+ subtypes. The statistical non-significance of that imbalance does not establish equivalence of baseline clinical profiles. Source estimates relied on a template head model, and SCD/SCS require further validation. TMS-EEG findings also require cautious interpretation because sensory responses and residual artifacts may contribute to evoked signals [18]. The exploratory correlations do not establish prediction or mediation, and only two assessment time points were available. Finally, no longer-term outcomes were assessed, so the durability of the behavioral and neurophysiological changes remains unknown.

Conclusions

Four weeks of taVNS was associated with greater improvement in behavioral responsiveness than tnVNS and with changes in the magnitude and spatial spread of TMS-evoked cortical activity. The findings justify further investigation of auricular neuromodulation in neurorehabilitation and of TMS-EEG as an exploratory treatment-response assessment. Larger trials with longer follow-up, robust neurophysiological quality control, and independent biomarker validation are needed before these measures can guide clinical decisions.

Abbreviations

ANOVA

analysis of variance
BEM
boundary element method
CI
confidence interval
CRS-R
Coma Recovery Scale-Revised
DOC
disorders of consciousness
EEG
electroencephalography
FDR
false discovery rate
GMFP
global mean field power
ICA
independent component analysis
MCS
minimally conscious state
RMT
resting motor threshold
SCD
significant current density
SCS
significant current scattering
SD
standard deviation
sLORETA
standardized low-resolution brain electromagnetic tomography
taVNS
transcutaneous auricular vagus nerve stimulation
TBI
traumatic brain injury
TEP
TMS-evoked potential
TMS
transcranial magnetic stimulation
TMS-EEG
transcranial magnetic stimulation-electroencephalography
tnVNS

transcutaneous non-vagal nerve stimulation
VNS
vagus nerve stimulation.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of the Institute of Acupuncture and Moxibustion, China Academy of Chinese Medical Sciences (S2024-01-31-13). Written informed consent was obtained from the legal representative of each participant.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and analysed during the current study are not publicly available because of participant privacy and institutional and ethical restrictions, but de-identified data are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

YoW (Yong Wang): investigation, data curation, formal analysis, visualization, and writing—original draft. TC (Tianqing Cao): participant recruitment, investigation, and data curation. JZ (Jinling Zhang): TMS-EEG acquisition, data preprocessing, and formal analysis. WZ (Weihang Zhai): data curation. DL (Dongsheng Liu): investigation, clinical assessment, and resources. YY (Yi Yang): conceptualization, methodology, supervision, and writing—review and editing. YfW (Yifei Wang): conceptualization, methodology, project

administration, funding acquisition, supervision, and writing—review and editing. All authors read and approved the final manuscript.

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Figures

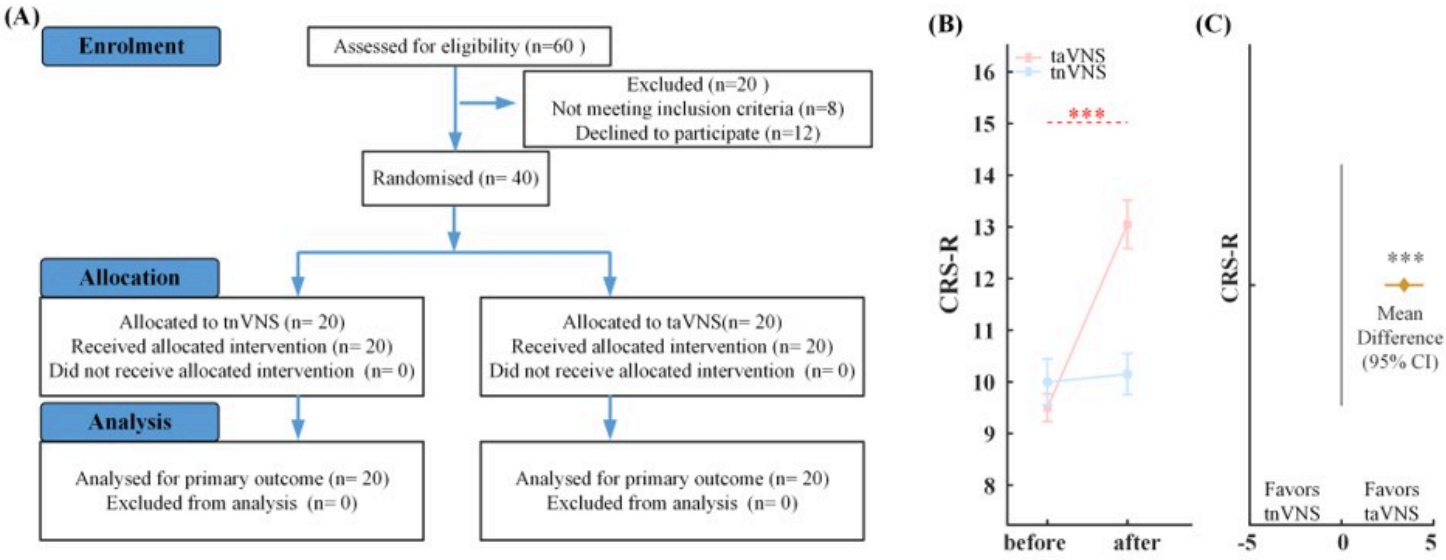


Figure 1

Trial profile and behavioral response.

(A) CONSORT flow diagram of enrolment, allocation, follow-up, and analysis. (B) CRS-R scores (mean ± SD) before and after intervention in the two groups. (C) Forest plot of the between-group difference in ΔCRS-R: the diamond indicates the mean difference, the horizontal line the 95% confidence interval, and the solid vertical line the line of no difference. ***p < 0.001.

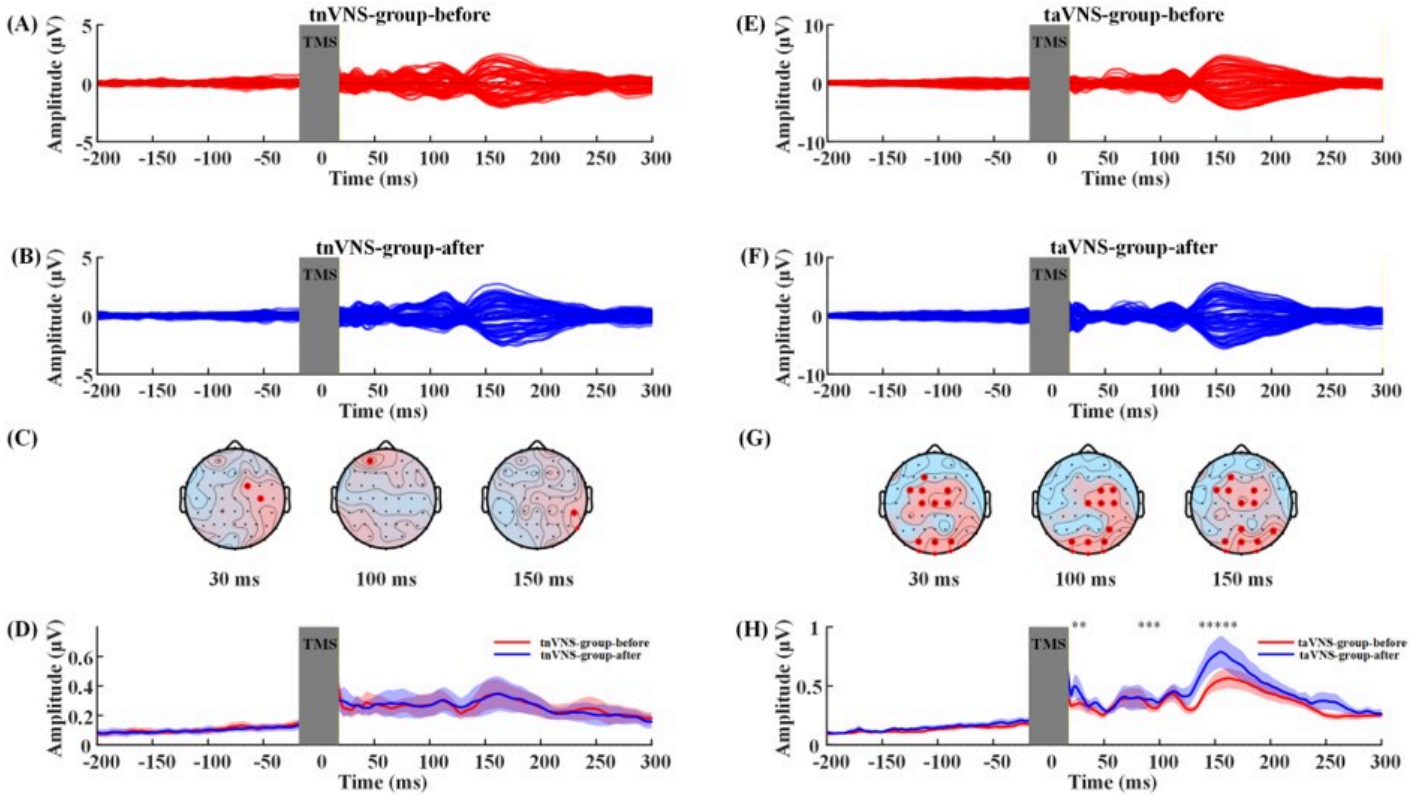


Figure 2

Scalp-level TMS-EEG responses.

(A, B) TEP butterfly plots before and after tnVNS. (E, F) Corresponding plots before and after taVNS. (C, G) Topographical maps at 30, 100 and 150 ms. (D, H) GMFP time courses for tnVNS and taVNS, respectively. Red and blue curves indicate before and after intervention. Gray regions mark the TMS-associated interval displayed in the original plots. Electrode and time-course markers indicate the within-group comparisons described in Methods.

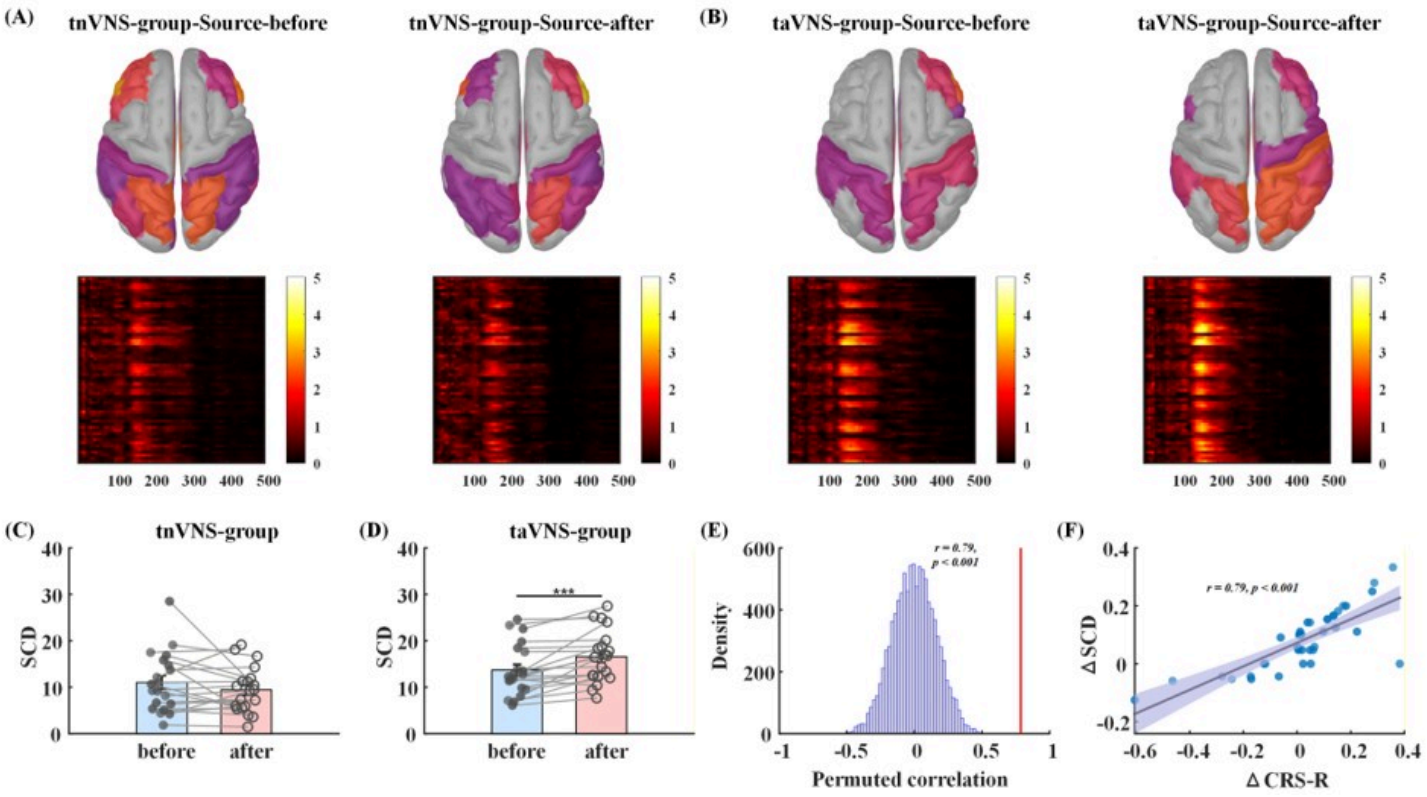


Figure 3

Source-level TMS-evoked responses.

(A, B) Cortical source maps and time-resolved source displays before and after tnVNS and taVNS, respectively. (C, D) Individual paired SCD values before and after intervention. (E) Null distribution from the permutation correlation analysis. (F) Association between SCD change and CRS-R change. Source reconstructions and statistical annotations are retained from the original analysis.

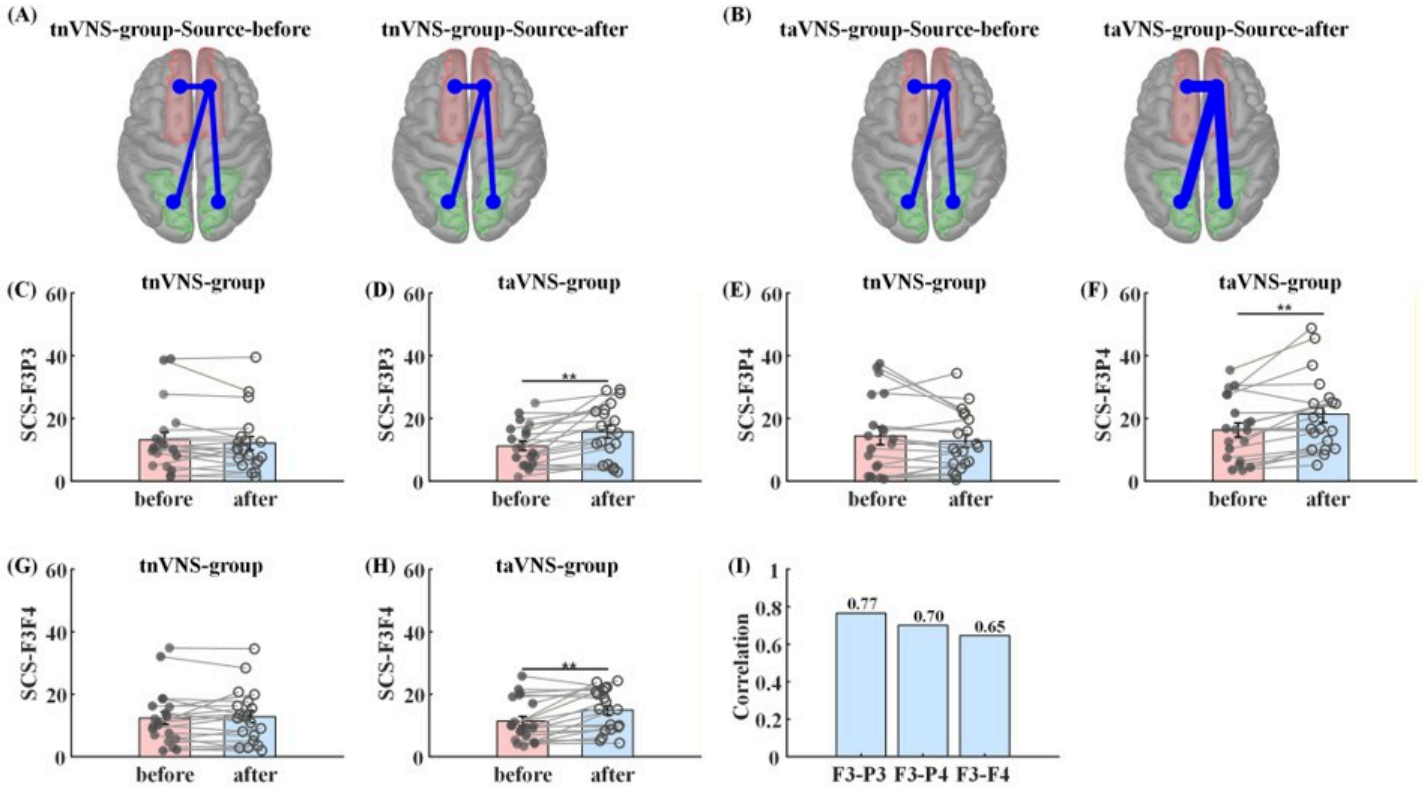


Figure 4

Spatial spread of TMS-evoked source activity.

(A, B) Schematic displays of the three region pairs before and after tnVNS and taVNS, respectively. (C, D) SCS for F3–P3. (E, F) SCS for F3–P4. (G, H) SCS for F3–F4. Paired points show individual pre-to-post values. (I) Reported correlations between SCS changes and CRS-R changes for the three region pairs. Lines in the schematic identify the region pairs analyzed and should not be interpreted as tractography.