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Rhythmic musical-electrical trigeminal nerve stimulation improves impaired consciousness

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ABSTRACT

Accumulating evidence shows that consciousness is linked to neural oscillations in the thalamocortical system, suggesting that deficits in these oscillations may underlie disorders of consciousness (DOC). However, patient-friendly non-invasive treatments targeting this functional anomaly are still missing and the therapeutic value of oscillation restoration has remained unclear. We propose a novel approach that aims to restore DOC patients' thalamocortical oscillations by combining rhythmic trigeminal-nerve stimulation with comodulated musical stimulation ("musical-electrical TNS"). In a double-blind, placebo-controlled, parallel-group study, we recruited 63 patients with DOC and randomly assigned them to groups receiving gamma, beta, or sham musical-electrical TNS. The stimulation was applied for 40 min on five consecutive days. We measured patients' consciousness before and after the stimulation using behavioral indicators and neural responses to rhythmic auditory speech. We further assessed their outcomes one year later. We found that musical-electrical TNS reliably lead to improvements in consciousness and oscillatory brain activity at the stimulation frequency: 43.5 % of patients in the gamma group and 25 % of patients in the beta group showed an improvement of their diagnosis after being treated with the stimulation. This group of benefitting patients still showed more positive outcomes one year later. Moreover, patients with stronger behavioral benefits showed stronger improvements in oscillatory brain activity. These findings suggest that brain oscillations contribute to consciousness and that musical-electrical TNS may serve as a promising approach to improve consciousness and predict long-term outcomes in patients with DOC.

1. Introduction

Neurological disease may be treated by restoring the specific brain functions impaired by the disease. In the case of disorders of consciousness (DOC), accumulating neuroscientific evidence shows a link between the unconscious state and a perturbation in neural oscillations in thalamic and cortical structures (Koch et al., 2016; Redinbaugh et al., 2020; Supp et al., 2011). For example, injection of propofol has been shown to induce both an unconscious state and a perturbation in neural oscillations in the thalamocortical system (Flores et al., 2017; Supp et al., 2011). Moreover, consciousness level in DOC patients and lucid dreaming in healthy subjects have been shown to correlate positively with the strength of oscillatory activity in the cerebral cortex specifically at gamma (~40 Hz) frequency (Binder et al., 2017; Cavinato et al., 2015; Gorska and Binder, 2019; Voss et al., 2014). Similarly, studies in healthy subjects have shown enhanced gamma-band synchrony in response to consciously perceived stimuli compared to unperceived stimuli (Gaillard et al., 2009; Melloni et al., 2007; Steinmann et al., 2014). According to

Abbreviations: ASSR, auditory steady-state response; CRS-R, Coma Recovery Scale-Revised; DOC, disorders of consciousness; EMCS, emergence from minimally conscious state; ERP, event-related potential; FOI, frequency of interest; GOSE, Glasgow Outcome Scale-Extended; ICA, independent component analysis; MCS, minimally conscious state; tACS, transcranial alternating current stimulation; TBI, traumatic brain injury; tDCS, transcranial direct current stimulation; TNS, trigeminal nerve stimulation; UWS, unresponsive wakefulness syndrome.

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the mesocircuit hypothesis, deficits in consciousness are caused by a global decrease of excitatory neurotransmission from the central thalamus to diffuse cortical areas (Schiff, 2010). However, despite the evidence for a correlation of consciousness and thalamocortical oscillations, in particular gamma oscillations, their causal link has not been fully established, leaving the therapeutic value of the oscillations unclear.

A safe and convenient method to potentially restore neural oscillations is transcranial alternating current stimulation (tACS) (Antal and Paulus, 2013; Jones et al., 2020; Naro et al., 2016). However, the neural effectiveness of tACS is currently debated based on findings showing that cortical effects may be an epiphenomenon of co-stimulation of peripheral nerves (Asamoah et al., 2019). Given these considerations, a more effective non-invasive approach to restore neural oscillations may be transcutaneous stimulation (Adair et al., 2020; Conlon et al., 2020), which involves the application of an electrical current to the skin directly above a peripheral or cranial nerve. The trigeminal nerve, as the largest human cranial nerve, has abundant anatomical projections to somatosensory nuclei of the thalamus in the midbrain (McCormick and Bal, 1994), making it a strong candidate for non-invasive modulation of neural oscillations in thalamocortical circuits. Application of trigeminal nerve stimulation (TNS) has yielded encouraging results in various neurological diseases, such as epilepsy, depressive disorder and migraine (DeGiorgio et al., 2013; Gorgulho et al., 2019; Reuter et al., 2019). For DOC, only a single study in a single patient has been published, with promising results (Fan et al., 2019). A systematic investigation of the potential of TNS for DOC treatment is still missing.

Besides electrical stimulation, neural oscillations may be restored with sensory stimulation. Application of rhythmic sensory stimulation induces modulations in neural oscillations specifically at the frequency of the stimulation (Iaccarino et al., 2016; Martorell et al., 2019; Pastor et al., 2002). Thus, rhythmic sensory stimulation at gamma frequency has been exploited to alleviate cognitive dysfunctions accompanied by abnormal neural gamma oscillations (Clements-Cortes et al., 2016; Iaccarino et al., 2016; Martorell et al., 2019). However, whether rhythmic sensory stimulation can restore DOC patients' pathological neural oscillations and consciousness has not been investigated yet.

Considering the key role of the thalamus in the regulation and integration of multisensory information (Sherman and Guillery, 1996), the most effective approach to target neural oscillations in multiple thalamic nuclei and thalamocortical circuits might be a combination of the aforementioned transcutaneous and sensory modalities. Indeed, a recent study in tinnitus patients shows stronger behavioral and neural effects induced by combined acoustic-electrical peripheral nerve stimulation than by acoustic stimulation alone (Marks et al., 2018).

Based on the findings and considerations above, we reasoned that combining rhythmic TNS and rhythmic musical stimulation ("rhythmic musical-electrical TNS" for simplicity) at gamma frequency could provide a highly effective approach to restore thalamocortical oscillations and consciousness in DOC patients. We predicted that the stimulation improves patients' consciousness and neural oscillations, which would render rhythmic musical-electrical TNS a strong candidate for DOC treatment. We further predicted that the putative behavioral and neural benefits are coupled, which would corroborate the notion that neural oscillations and consciousness are functionally coupled. Finally, we predicted that patients who directly benefitted from the stimulation would show a more successful long-term recovery.

2. Methods

2.1. Patients

Seventy-two patients diagnosed with DOC were recruited from Hangzhou Mingzhou Brain Rehabilitation Hospital. All patients met the following criteria: (1) diagnosis of unresponsive wakefulness syndrome (UWS, a state showing only reflex movements) or minimally conscious state (MCS, a state showing reproducible but inconsistent signs of consciousness) based on the Coma Recovery Scale-Revised (CRS-R), (2) time post-injury ranging from one to twelve months, (3) no history of psychiatric or neurological diseases, (4) no major skull-bone defects and no major brain-tissue defects (approximately $>$ 70 % of total brain volume preserved, based on visual inspection of anatomical computed-tomography or magnetic-resonance images), and (5) no history of hearing impairment before brain injury. The study was approved by the local research-ethics committee (Hangzhou Mingzhou Brain Rehabilitation Hospital) and registered in a publicly accessible clinical trial database on www.clinicaltrials.gov (NCT04435301). Informed consent was obtained from the patients' legal surrogates. The patients were divided into three groups differing in the type of stimulation they received. To reduce the risk of confounding effects of relevant demographic characteristics (i.e., age, etiology, and time since injury) or consciousness level (i.e., CRS-R total score and diagnosis) before the experiment, the groups were matched for the aforementioned variables as much as possible. The matching procedure consisted of three steps: first, we dichotomized each matching variable and scored it as "1" or "0", where a score of 1 indicates: age $<$ 60, etiology = traumatic brain injury (TBI), time since injury $<$ 3 month, and CRS-R score $>$ 8, respectively. Second, based on these scores, we divided the 72 recruited patients into 24 triplets. As not all patients could be matched on their exact pattern of scores, we matched them on their sum of scores. Finally, we randomly assigned the three patients within each triplet to the three stimulation groups. Sixty-three patients (42 MCS, 21 UWS) completed the study (six patients were released from the hospital and three others showed excessive body movements during the pretest phase, Fig. S1). These patients' demographic and clinical characteristics are listed in Table S1 and Table S2, showing that the three groups did not differ systematically in any of the matching variables.

2.2. Study design and experimental procedure

The study utilized a double-blind, placebo-controlled, parallel-group design, where each participant received gamma, non-gamma (beta) or sham stimulation. The beta frequency was selected as a control frequency of gamma. Each patient underwent four consecutive phases (Fig. 1B). During the pretest phase, behavioral assessments were administrated on five consecutive days with a single neural assessment administered on the fifth day. During the stimulation phase, stimulation was administrated on each of five consecutive days, starting on the first day after the pretest phase. During the posttest phase, the same set of behavioral and neural assessments was administrated as during the pretest phase, except that the neural assessment took place already on the first day after the stimulation phase. Finally, during the follow-up phase, a single behavioral assessment of patients' one-year outcome was administrated. Researchers were blinded to the stimulation conditions by replacing the labels of stimulation conditions with dummy codes. Patients and their legal surrogates were blinded to the type of stimulation applied to the patient.

2.3. Assessment

The pretest phase and posttest phase involved the administration of the same set of behavioral and neural assessments, as illustrated in Fig. 1A top. The follow-up phase involved the administration of a single behavioral assessment of outcome.

2.3.1. Behavioral assessment: CRS-R and GOSE

Patients' consciousness was assessed using the CRS-R, a validated measure of consciousness that currently constitutes the golden standard for behavioral diagnosis of DOC patients (Giacino et al., 2004; Giacino et al., 2018b; Kondziella et al., 2020). The CRS-R consists of six subscales assessing respectively the patient's auditory function, visual function, motor function, oromotor function, communication ability, and arousal.

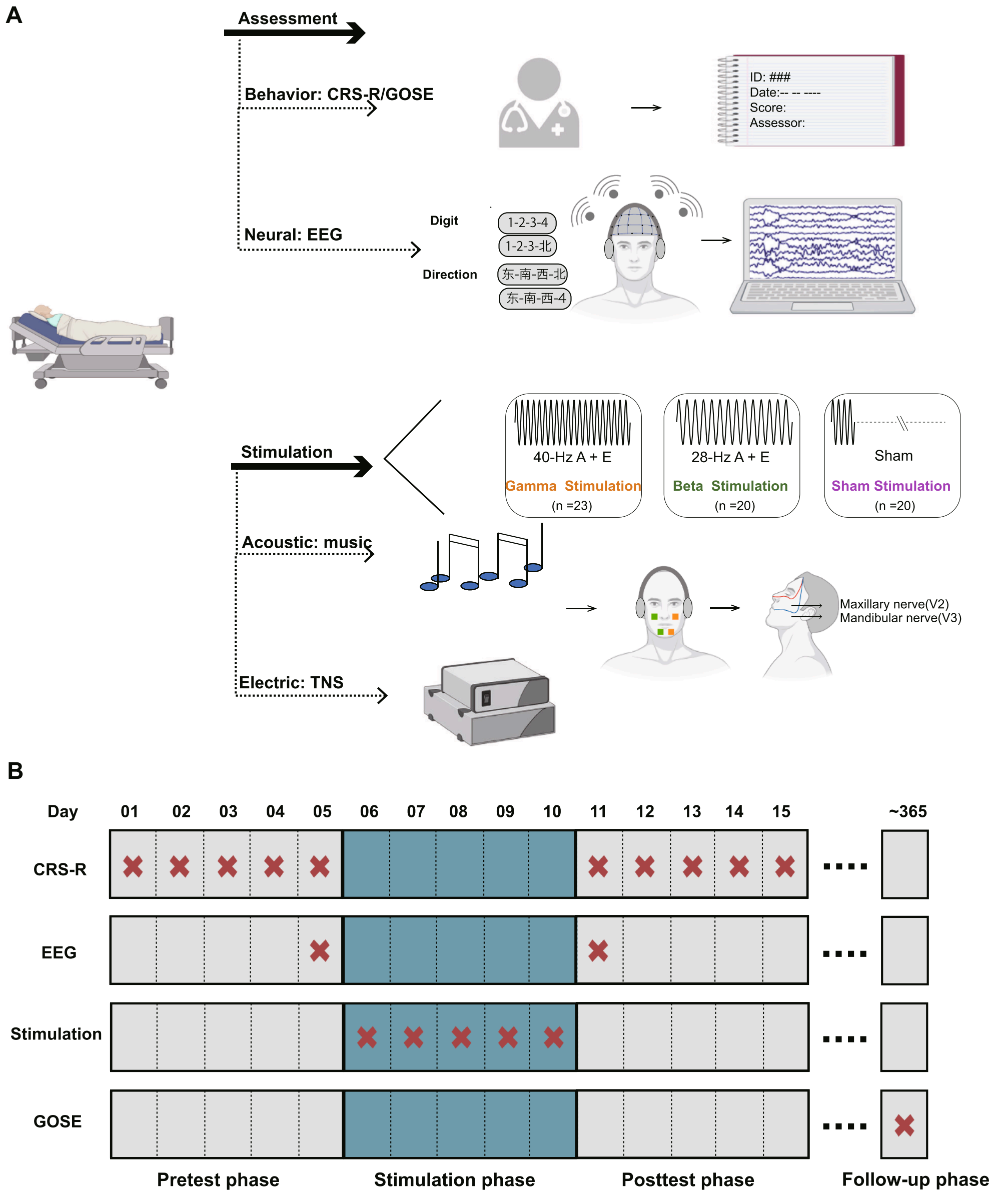

Fig. 1. Study design and experimental procedure. (A). Schematic of assessments and musical-electrical TNS. DOC patients' consciousness was assessed by two experienced blinded clinicians using the CRS-R. Long-term outcomes were assessed using the GOSE. Patients' brain activity was assessed by measuring 64-channel EEG during the presentation of continuous rhythmic auditory Chinese speech. The rhythmic musical-electrical TNS involved the simultaneous application of auditory music via earphones and electrical current via electrodes attached to the middle and lower parts of the patient's face (see green and orange squares). Both acoustic and electrical stimulation were amplitude-modulated at gamma frequency (40 Hz, gamma-stimulation group) or beta frequency (28 Hz, beta-stimulation group), or their intensities were set to zero after a short (30 s) initial stimulation interval (sham-stimulation group). (B). Experimental procedure. Each patient underwent a 15-day long experimental procedure consisting of three 5-day long consecutive phases (pretest, stimulation, and posttest,) and a follow-up assessment approximately-one year after the initial CRS-R assessment. The treatment phase involved daily administration of 40 min of rhythmic musical-electrical TNS or sham stimulation. The pretest and posttest phases involved administration of daily behavioral assessments (CRS-R) and a single neural assessment (EEG) on the day immediately before and after the stimulation phase, respectively. The follow-up phase involved administration of a single behavioral assessment (GOSE). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Each subscale comprises several hierarchically arranged items (23 in total) allowing for a quantitative assessment. Based on the subscale scores, the patient is diagnosed as either UWS, MCS (including two subcategories, termed MCS+ and MCS-), or emergence from MCS (EMCS). To reduce the risk of misdiagnosis, the CRS-R was administered twice a day by two experienced clinicians on five consecutive days (Wannez et al., 2017); thus, each patient underwent the same number of CRS-R administrations. The patient's overall diagnosis was defined as the "best" diagnosis (i.e., highest level of consciousness) observed across the daily measurements. The patient's overall CRS-R score was defined as the sum of the subscores obtained during that best measurement. An improvement of diagnosis was defined as an upgrade of diagnosis from pretest to posttest phase (i.e., UWS to MCS/EMCS, MCS- to MCS+/EMCS, or MCS+ to EMCS).

Patients' long-term outcomes were assessed with the Glasgow Outcome Scale-Extended (GOSE), a widely used index for outcomes after brain injury (Wilson et al., 1998). It consists of eight categories rating patients' state from *dead* to *upper good recovery*. The GOSE was obtained by interviewing patients' caregivers and having audio recordings of the interviews evaluated by an experienced clinician who was blinded to the study design. Thirty-six out of the 43 patients who had received the verum stimulation (i.e., gamma and beta groups, see below section Stimulation) were available for this follow-up assessment (Fig. S1), which was conducted approximately-one year (11.9 ± 0.5 months, mean ± SD across patients) after the initial CRS-R assessment.

2.3.2. Neural assessment: EEG

2.3.2.1. EEG recording. Patients' brain activity was assessed during the presentation of auditory stimuli (see next section) using 64 active scalp EEG electrodes (BrainCap, Brain Products, Munich, Germany) placed according to a standard 10–20 system. An additional electrode was placed at the suborbital ridge to record the electrooculogram. Position FCz was used for the reference electrode. Impedances were kept below 10 kΩ. The EEG recordings were online bandpass-filtered between 0.01 Hz and 70 Hz, and digitized with a sampling rate of 1 kHz.

2.3.2.2. Auditory paradigm for neural assessment. A novel auditory paradigm with speech stimuli was used to facilitate assessment of neural rhythmic activity and speech processing. The auditory-speech paradigm (Fig. 1A top, 1B) was inspired by previous work on speech processing

(Ding et al., 2016; Gui et al., 2020). The speech stimuli were repetitive word quartets of isochronous amplitude-modulated words. Eight monosyllabic Chinese words were used: *one, two, three, four, east, south, west, north*. To avoid systematic prosody differences across words, each word was synthesized individually using a freely available text-to-speech engine (female voice, Duxiaomei, http://ai.baidu.com/tech/speech/tts).

To elicit neural tracking of hierarchical linguistic structures, word duration was fixed to 0.5 s (by inserting silent intervals at the beginning and end of each word) and words were sequenced into one of two semantically congruent word quartets (*"one-two–three-four"* or *"east-south-west-north"*, which is the conventional order in Chinese). We refer to these word quartets as "digit sentence" or "direction sentence" for simplicity. Each sentence was periodically presented sixteen times to form a 32-s long continuous sequence, which constituted a single trial. As a result, the overall stimuli carried two hierarchical linguistic structures, one at the word rate (2 Hz) and the other at the sentence rate (0.5 Hz).

To elicit neural detection of semantic violations, a semantically incongruent word was inserted in three to five pseudorandomly chosen sentences per trial, excluding the first and last sentence. This was done by swapping the final word of the chosen sentence (e.g., *"four"* in the digit sentence) with the final word of the other type of sentence (e.g., *"north"*, or vice versa), resulting in "*one-two–three-north*" or *"east-south-west-four*". To elicit rhythmic brain activity at beta or gamma frequency, each word was amplitude-modulated at a frequency of 28 Hz or 40 Hz (sinusoidal modulation, depth: 100 %, fixed starting phase). Auditory stimuli were delivered diotically through insert earphones at a fixed sound level (62 dB SPL) using e-prime software (Psychology Software Tools, Inc., Pittsburgh, PA, USA). Patient 49 was accidentally presented with an inappropriate sound level (10 dB SPL) at posttest and therefore excluded from the neural data analysis.

Trials were presented in blocks of 20. Half of these trials contained speech carrying the 40-Hz amplitude modulation and the other half involved the 28-Hz amplitude modulation. Trials within blocks were randomly sequenced with a jittered inter-trial interval of 1.5 to 2 s. Each patient was presented with four consecutive blocks each lasting approximately 11.5 min. Odd-numbered blocks contained only trials constructed from the digit sentence and even-numbered blocks contained only trials constructed from the direction sentence. Each block started with a brief synthesized audio message, which instructed patients to listen carefully to the upcoming speech. To ensure that patients could perceive the task instructions, the experimenters monitored patients' state of wakefulness before and during each block. Patient 23 was observed to fall asleep after the first block in posttest and therefore excluded from the neural data analysis.

2.4. Stimulation

The stimulation phase involved administration of rhythmic musical-electrical stimulation or sham stimulation to the patients' face and ears, as illustrated in Fig. 1A bottom.

2.4.1. Acoustic stimulation: Music

Acoustic stimulation consisted of excerpts from ten pieces of popular Chinese music. The onset/offset of each excerpt was ramped up/down using 5-s long ramps. Excerpts were amplitude-compressed (compression ratio: 120:1, threshold: −12 dB) and sequenced to form a continuous 40-min long stream of music. To enforce rhythmic brain activity at gamma or beta frequency, the sequence was amplitude-modulated at a frequency of 40 Hz or 28 Hz (gamma stimulation and beta stimulation, respectively; sinusoidal modulation, depth: 100 %). The amplitude of the overall sequence was scaled to avoid clipping. The acoustic stimulation was presented diotically through insert earphones at a fixed sound level (50 dB SPL) simultaneously with the electrical or sham stimulation (see next section).

2.4.2. Electrical stimulation: TNS

Electrical stimulation consisted of non-invasive application of alternating currents to patients' face to facilitate rhythmic trigeminal nerve activity. Analogously to the acoustic stimulation, the current waveform was a sinusoid with a frequency of 40 Hz or 28 Hz (gamma stimulation and beta stimulation, respectively). The current was applied using two pairs of square-shaped rubber electrodes (size: 3 cm × 3 cm) placed at the bilateral middle and lower part of the patient's face to stimulate the second and third branches of the trigeminal nerve (i.e., the maxillary nerve and the mandibular nerve, respectively); see Fig. 1A bottom. These stimulation sites were deemed to reduce phosphenes (compared with stimulation at the first branch) based on prior tests with healthy participants. The intensity of the current was fixed to ± 8 mA, which can be considered safe based on the aforementioned prior tests, related TNS research (Fan et al., 2019), and our *post hoc* observation of no skin condition after the stimulation phase in any of our patients. The onset/offset of the current was ramped up/down using 5-s long ramps. The electrodes were adhered to patients' skin using conductive paste and the impedance was kept below 5 kΩ. The electrical stimulation was delivered continuously for 40 min using a battery-powered DC stimulator (DCSTIMULATOR MC, NeuroConn, Germany).

Sham stimulation was identical to the gamma stimulation above, except that the musical-electrical stimulation was ramped down after 0.5 min to remain turned off for the remaining 39.5 min (Kasten and Herrmann, 2017).

2.5. Data analysis

2.5.1. EEG data preprocessing

Data preprocessing and analysis was performed offline using EEGLAB 2019.1 (Delorme and Makeig, 2004) and MATLAB 9.4. First, bad channels with a leptokurtic voltage distribution (i.e., kurtosis higher than five) were replaced by interpolating between the surrounding electrodes (spherical spline interpolation; percentage of interpolated channels: 3.8 ± 3.4, mean ± SD across patients). Second, the interpolated channel data were re-referenced to an average reference. Third, to reduce artifacts, independent component analysis (ICA) was applied to the channel data using a second-order blind-identification algorithm (Belouchrani et al., 1997). For this analysis the data were band-pass filtered between 1 Hz and 40 Hz using a linear-phase finite impulse response filter (zero phase shift, filter order: 3300). Artifactual components were identified and discarded (percentage of artifactual components: 15.5 % ± 9.1 %; mean ± SD across patients) using the EEGLAB plugin ICLables (Pion-Tonachini et al., 2019). The weights of the non-artifactual components were reapplied to the original unfiltered channel data (Jaeger et al., 2018). Patient 29 was observed to show excessively high noise levels resulting in an abnormally high number of artifacts (more than three SDs above the mean) and therefore was excluded from further neural data analysis. Fourth, only for the analysis of event-related potentials (ERPs, see below), the ICA-cleaned channel data were band-pass filtered as above using cutoff frequencies 0.5 Hz and 20 Hz. Finally, the continuous channel data were segmented into 30-s epochs resembling single trials (the first sentence interval was rejected from each trial to avoid onset effects).

2.5.2. Analysis of rhythmic brain activity

For the analysis of rhythmic brain activity, epochs were averaged separately for trials containing the 40-Hz or 28-Hz amplitude modulation. To assess rhythmic brain activity at these frequencies of interest (FOIs), a discrete Fourier transform was applied (30000 points, resulting in a frequency resolution of 0.03 Hz/bin). The auditory steady-state response (ASSR) was calculated by dividing the spectral amplitude at each FOI by the noise floor (calculated as the average amplitude of the ten bins on each side of the FOI). The resulting ratio was expressed on a dB scale and averaged across all EEG channels.

2.5.3. Analysis of neural tracking of hierarchical linguistic structures

Neural tracking of hierarchical linguistic structures was assessed as described in the preceding section, except for the following differences: first, trials containing different amplitude-modulation frequencies were pooled. Second, FOIs were defined as the sentence rate (0.5 Hz) and word rate (2 Hz). Finally, a narrower noise floor spanning only three bins on each side of the FOI was used.

2.5.4. Analysis of neural detection of semantic violations

For the analysis of semantic violation detection, epochs were further segmented into sub-epochs resembling the final 500-ms interval (i.e., the final word) in each sentence. Sub-epochs were normalized by subtracting a baseline (defined as the average amplitude within −100 to 0 ms relative to the onset of the sub-epoch) and averaged separately for trials containing a semantically congruent or incongruent word. The ERPs were averaged across centroparietal scalp regions (positions Cz, C1, C2, C3, C4, Pz, P1, P2, P3, P4, CPz, CP1, and CP2) that have been associated with semantic violation detection in previous ERP research (Kallionpaa et al., 2018). To identify time windows of semantic violation detection, ERP difference waveforms were computed by subtracting the ERP waveform to semantically incongruent words from the ERP waveform to semantically congruent words.

2.5.5. Extraction of an unbiased measure of the musical-electrical TNS effect

To extract the effect of the stimulation on patients' consciousness and brain activity, behavioral and neural data obtained during the posttest were subtracted from those obtained during the pretest, which was done for each stimulation group. To obtain an unbiased, straightforward measure of the effect of the actual rhythmic musical-electrical TNS (excluding potential effects of spontaneous recovery and/or placebo), the average change observed in the sham-stimulation group was subtracted from the individual pretest–posttest changes observed in the gamma- and beta-stimulation groups (Avins et al., 2012; Boucher et al., 2021).

2.6. Statistical analysis

Patients' individual behavioral and neural measures were submitted to second-level group analyses. For within-subject comparisons, statistical tests for repeated measures were used and for between-subject comparisons, statistical tests for independent measures were applied. Assumptions of normality and equal variance were verified respectively with Shapiro-Wilk tests and Levene's tests. For datasets with a distribution deviating significantly from a normal distribution, non-parametric statistical tests were used (Wilcoxon signed-rank tests for dependent samples, Wilcoxon rank-sum tests for independent samples); this applied only to the set of CRS-R scores shown in Fig. 2. For all other datasets, parametric statistical tests (ANOVAs and t-tests) could be used. The rank correlation between behavioral and neural stimulation effects and its significance was assessed using Spearman's correlation coefficient ρ. A significance criterion $\alpha = 0.05$ was used and type-I error probabilities inflated by multiple comparisons were corrected by controlling the false-discovery rate. For the identification of time windows of semantic violation detection, non-parametric statistics (based on 1000 permutations) and a multiple comparison correction based on a temporal cluster-size criterion were used (Maris and Oostenveld, 2007).

2.7. Ordinal logistic regression analysis

To test whether patients' benefits from rhythmic musical-electrical TNS have prognostic value for their one-year outcomes, we performed an ordinal logistic regression analysis including the GOSE as a response variable and the following predictor variables: the patients' diagnostic improvement (i.e., whether the patient was a responder vs non-responder to the rhythmic musical-electrical TNS) and four potentially recovery-relevant patient characteristics at the time of the pretest, i.e., age, etiology, time since injury, and CRS-R total score. The two categorical predictor variables (response to stimulation and etiology) were dummy-coded.

3. Results

3.1. Positive aftereffect of rhythmic musical-electrical TNS on patients' consciousness

We found a significant increase in patients' CRS-R total score after treatment with rhythmic TNS (gamma stimulation: $Z = 3.463$, $p = 5.3 \times 10^{-4}$, effect size $r = 0.511$; beta stimulation: $Z = 2.601$, $p = 0.009$, $r = 0.411$, Fig. 2A): ten out of 23 (43.5 %) and five out of 20 (25 %) patients showed an improvement of diagnosis (e.g., from UWS to MCS) after gamma and beta TNS, respectively (Fig. 2B). These results suggest a positive aftereffect of gamma and beta musical-electrical TNS on DOC patients' consciousness.

In contrast to the stimulation groups above, we found no significant improvement in CRS-R score or diagnosis after vs before sham stimulation ($Z = 0.175$, $p = 0.861$, $r = 0.028$, Fig. 2A-B). This non-significant CRS-R score change was significantly smaller than the change observed after gamma or beta stimulation (gamma vs sham: $Z = 3.291$, $p = 4.985 \times 10^{-4}$, $r = 0.502$; beta vs sham: $Z = 2.407$, $p = 0.008$, $r = 0.381$). These results suggests that the consciousness improvements observed above were caused by the actual musical-electrical TNS (and associated neural changes), rather than spontaneous recovery and/or a placebo effect.

To obtain an unbiased measure of the actual musical-electrical TNS effect (excluding any effect of spontaneous recovery and/or placebo), we corrected the stimulated patients' pretest–posttest changes for the changes observed after sham stimulation (See Methods). As shown in Fig. 2C, this unbiased effect was significantly larger than zero for each gamma and beta stimulation (gamma stimulation: $Z = 4.004$, $p = 6.2 \times 10^{-4}$, $r = 0.590$; beta stimulation: $Z = 3.039$, $p = 0.002$, $r = 0.481$). The effect of gamma stimulation was on average stronger than that of beta stimulation; however, this difference was not statistically significant ($Z = 2.092$, $p = 0.275$, $r = 0.167$, Fig. 2C). Overall, the behavioral results indicate that DOC patients' consciousness can be improved—beyond spontaneous recovery and/or placebo-related change—with rhythmic musical-electrical TNS at beta and especially gamma frequency.

Further exploratory analysis of individual CRS-R subscales revealed that the consciousness improvements concerned primarily patients' visual abilities (gamma stimulation: $Z = 4.318$, $p = 9.6 \times 10^{-5}$, $r = 0.637$; beta stimulation: $Z = 3.113$, $p = 0.006$, $r = 0.492$) and motor abilities (gamma stimulation: $Z = 3.654$, $p = 0.0008$, $r = 0.539$; beta stimulation: $Z = 3.499$, $p = 0.003$, $r = 0.553$) and to a lesser degree communication abilities (gamma stimulation: $Z = 2.460$, $p = 0.028$, $r = 0.363$; beta stimulation: $Z = 2.000$, $p = 0.092$, $r = 0.316$), but not auditory abilities, oromotor abilities, or arousal (auditory in gamma stimulation group: $Z = 2.094$, $p = 0.054$, $r = 0.309$; all other $p > 0.1$, FDR corrected, Fig. 2D). These results show that rhythmic musical-electrical TNS benefits primarily patients' visual and motor abilities.

3.2. Positive frequency-specific aftereffect of rhythmic stimulation on brain activity

To identify the brain processes that putatively mediated the observed effect of rhythmic musical-electrical TNS on DOC patients' consciousness, we next explored the effect of the stimulation on patients' rhythmic cortical activity evoked by rhythmic auditory speech, as measured with the ASSR.

We first observed that beta- and gamma-modulated speech evoked robust beta and gamma cortical responses (ASSR at 28 Hz and 40 Hz; see Methods) in each group during the pretest (spectral peak > noise floor: $p < 0.05$ for all groups and both ASSR frequencies; one-tailed paired *t*-test, FDR-corrected; Fig. 3A-C, left and center) and these responses were

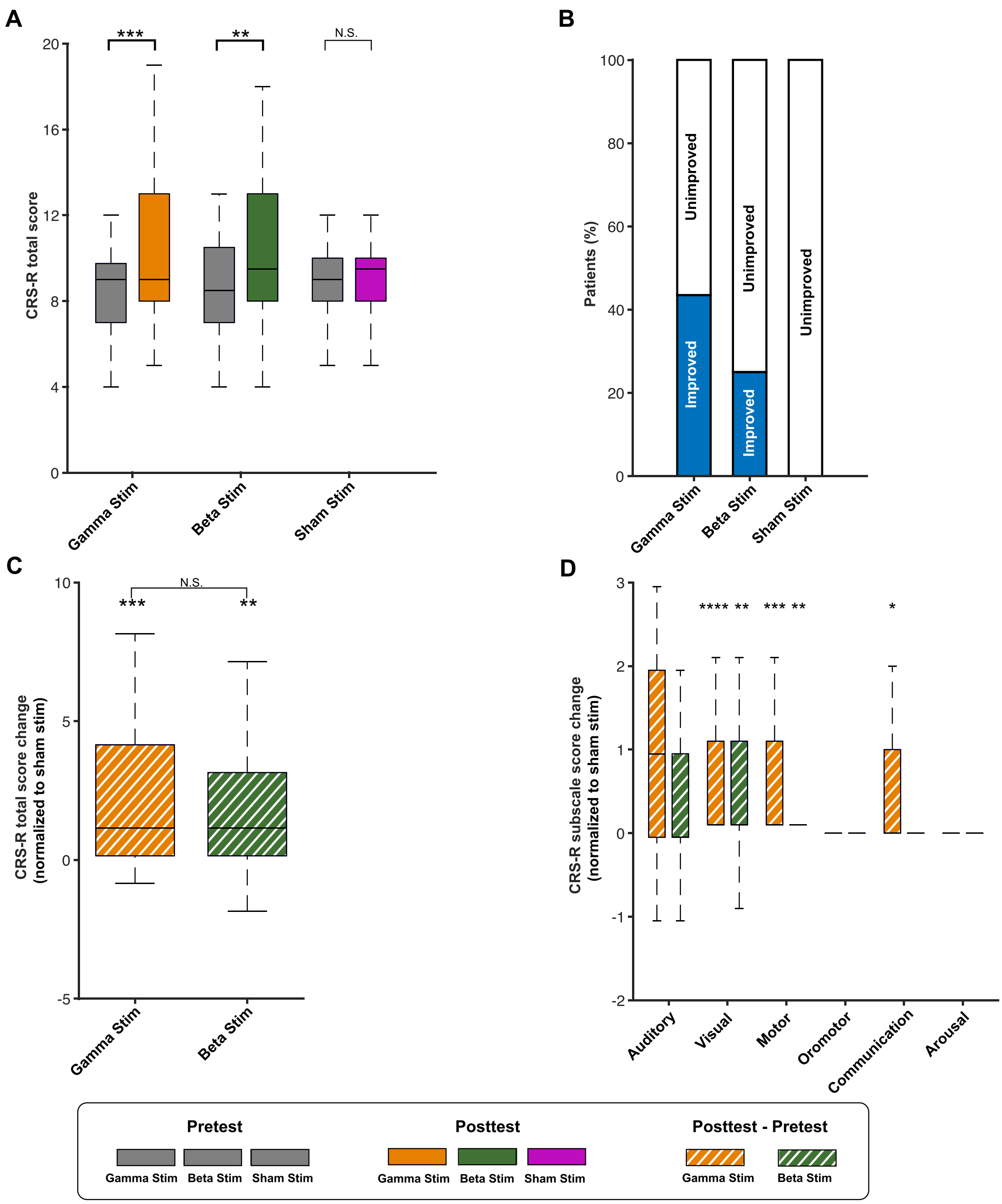

Fig. 2. Consciousness level in DOC patients before and after rhythmic musical-electrical TNS or sham stimulation. (A). CRS-R total scores of DOC patients in pretest (gray) and posttest (hue). The leftmost pair of boxes represents the consciousness level of the group of DOC patients who underwent gamma stimulation (gamma-stimulation group). The two other pairs represent results of matched patient groups receiving beta stimulation (beta-stimulation group) and sham stimulation (sham-stimulation group). Gamma stimulation and beta stimulation, but not sham stimulation, had positive effects on patients' consciousness. (B). Percentage of DOC patients showing improved diagnosis (blue) after gamma stimulation (left), beta stimulation (center), or sham stimulation (right). Gamma stimulation and beta stimulation, but not sham stimulation, had positive effects on patients' diagnosis. Overall, approximately-one third of the patients who received rhythmic musical-electrical TNS showed improved diagnosis. (C). Estimated effect of gamma stimulation (left) and beta stimulation (right) on patients' consciousness level (after correcting for sham stimulation-related changes; see Methods). Beta stimulation and especially gamma stimulation had positive effects on patients' consciousness, beyond spontaneous recovery or placebo-related changes. (D). Same as panel C, but stratified according to CRS-R subscales. Gamma stimulation (orange) and beta stimulation (green) improved patients' visual and motor abilities and to a lesser degree communication ability. Gamma stimulation also induced a change in auditory ability in only a small subset of patients; thus, this change was insignificant at the group level (corrected $p = 0.054$, non-parametric test). The horizontal line within each box in panels A, C, and D indicates the median value; the bottom and top edges of the box indicate the first and third quartile values; the whiskers indicate the most extreme values within 1.5 times the interquartile range. N.S. non-significant, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

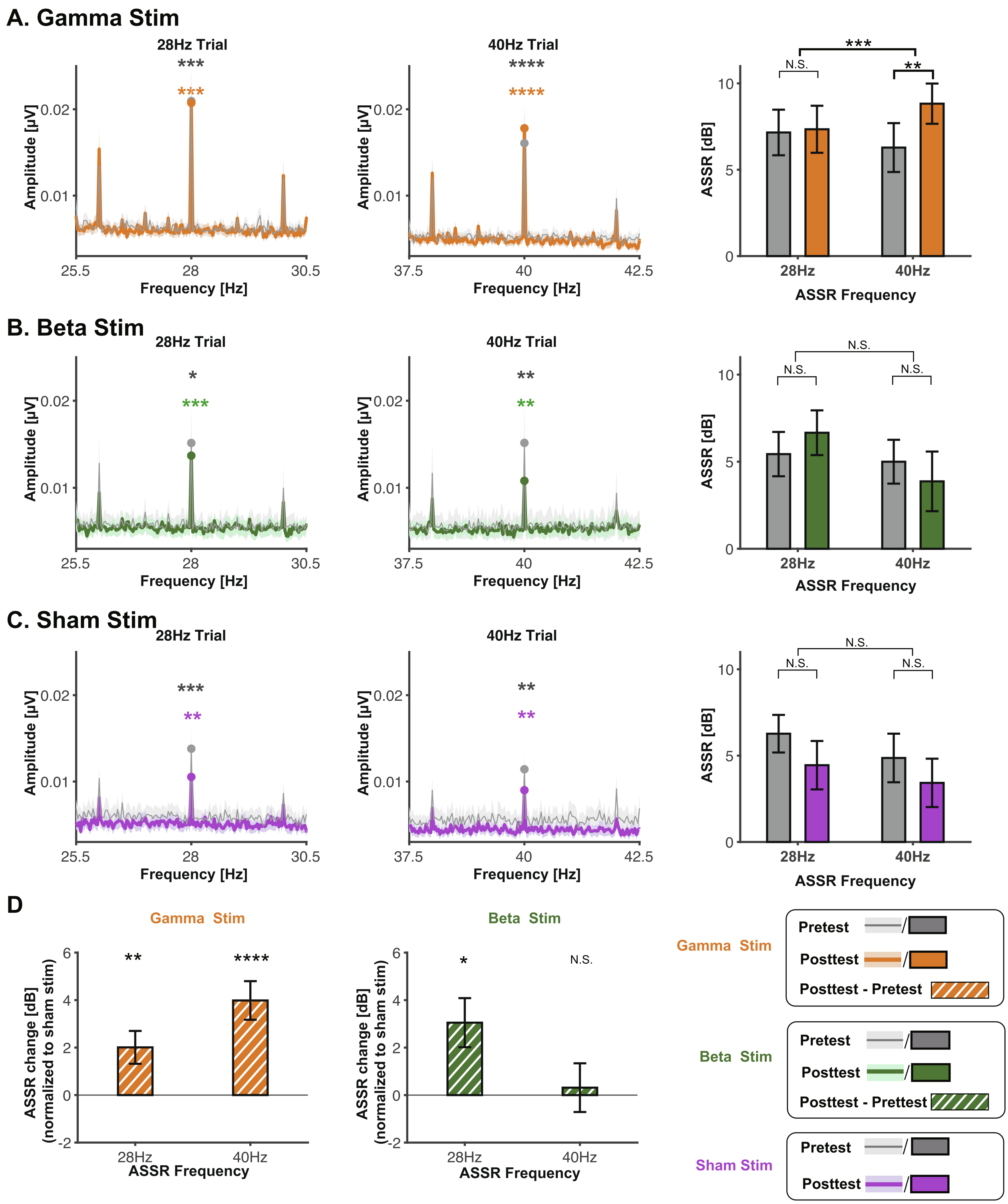

Fig. 3. Rhythmic brain responses to amplitude-modulated speech in DOC patients before and after rhythmic musical-electrical TNS or sham stimulation. (A). Spectral response to beta (28 Hz, left) or gamma (40 Hz, center) amplitude-modulated speech in pretest (gray) and posttest (orange) for the gamma-stimulation group. Asterisks indicate significant responses at the test frequency relative to the noise floor (average responses in the surrounding frequency bins), reflecting robust phase-locked cortical responses to the amplitude modulation of the auditory speech stimuli (one-tailed paired *t*-test, FDR-corrected). The two peaks surrounding the test frequency arise from the isochronous nature of the speech stimuli, which had a word rate of 2 Hz (see Methods). The bar plot on the right shows the ASSR (amplitude at test frequency relative to noise floor), a measure of rhythmic brain activity, in pretest and posttest. It can be seen that ASSR at gamma frequency was significantly enhanced after vs before gamma stimulation. No such effect is observable on ASSR at beta frequency. (B). Same as (A) but for the beta-stimulation group. (C). Same as (A) but for the sham-stimulation group. (D). Estimated effect of gamma stimulation (left plot) and beta stimulation (right plot) on beta ASSR and gamma ASSR, after correcting for sham stimulation-related changes (see Methods). Asterisks represent that unbiased neural changes after stimulation are significantly larger than zero, suggesting that gamma stimulation and beta stimulation had significantly positive effects on rhythmic brain activity especially at the stimulation frequency (two-tailed paired *t*-test). Data are presented as mean ± sem across participants. n.s. non-significant, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

of similar strength in the three groups (no effect of *stimulation type*: gamma ASSR: $F_{2,59} = 0.324$, $p = 0.725$, effect size $\eta_p^2 = 0.011$; beta ASSR: $F_{2,59} = 0.466$, $p = 0.630$, $\eta_p^2 = 0.016$; one-way between-subjects ANOVAs with factor *stimulation type* [gamma stimulation, beta stimulation, sham stimulation]).

Importantly, comparison of these rhythmic cortical responses after vs before stimulation revealed a significant positive effect of gamma stimulation on gamma responses ($t_{21} = 3.056$, $p = 0.006$, effect size $d = 0.652$; Fig. 3A, right) but not beta responses ($t_{21} = 0.262$, $p = 0.796$, $d = 0.056$), with a significantly stronger effect on gamma responses than beta responses (interaction *time* [pretest, posttest] × *ASSR frequency* [beta, gamma]: $F_{1,21} = 15.557$, $p = 7.4 \times 10^{-4}$, $\eta_p^2 = 0.426$). For beta stimulation, we found no significant effect on beta responses ($t_{18} = 1.155$, $p = 0.263$, $d = 0.265$, Fig. 3B, right) or gamma responses ($t_{18} = 1.071$, $p = 0.298$, $d = 0.246$) and a trend towards an interaction *time* × *ASSR frequency* ($F_{1,18} = 3.810$, $p = 0.067$, $\eta_p^2 = 0.175$). The latter beta stimulation-induced trend mirrored the above gamma stimulation-induced interaction, suggesting that the rhythmic musical-electrical TNS facilitated rhythmic brain activity specifically at the stimulation frequency.

The sham-stimulation group showed a reduction in beta and gamma responses that was not statistically significant (beta ASSR: $t_{18} = 1.479$, $p = 0.156$, $d = 0.339$; gamma ASSR: $t_{19} = 1.241$, $p = 0.231$, $d = 0.285$; interaction *time* × *ASSR frequency*: $F_{1,18} = 0.091$, $p = 0.767$, $\eta_p^2 = 0.005$, Fig. 3C, right). The non-significant effect of sham stimulation on gamma responses was significantly smaller than the effect of gamma, but not beta, stimulation (interaction *time* × *stimulation type* [gamma stimulation, sham stimulation]: $F_{1,39} = 8.077$, $p = 0.004$, $\eta_p^2 = 0.172$; interaction *time* × *stimulation type* [beta stimulation, sham stimulation]: $F_{1,36} = 0.040$, $p = 0.421$, $\eta_p^2 = 0.001$). Mirroring this pattern, the non-significant effect of sham stimulation on beta responses was significantly smaller than the effect of beta, but not gamma, stimulation (interaction *time* × *stimulation type* [beta stimulation, sham stimulation]: $F_{1,36} = 3.515$, $p = 0.024$, $\eta_p^2 = 0.089$; interaction *time* × *stimulation type* [gamma stimulation, sham stimulation]: $F_{1,39} = 2.136$, $p = 0.076$, $\eta_p^2 = 0.052$).

To obtain an unbiased measure of the musical-electrical TNS effect, we corrected the stimulated patients' neural changes for the observed average sham stimulation-related change as described above (see behavioral results and Methods). Fig. 3D (left) shows that the unbiased neural effect of gamma stimulation was significantly larger than zero, resulting in enhancement of beta responses (average increase: 2.0 dB; $t_{21} = 2.836$, $p = 0.009$, $d = 0.605$) and especially gamma responses (average increase: 4.0 dB; $t_{21} = 4.790$, $p = 9.9 \times 10^{-5}$, $d = 1.021$). Similarly, we observed an enhancing effect of beta stimulation on beta responses (average increase: 3.0 dB; $t_{18} = 2.872$, $p = 0.010$, $d = 0.656$; Fig. 3D right), but not gamma responses (average increase: 0.3 dB; $t_{18} = 0.298$, $p = 0.769$, $d = 0.068$). Pooled across the two stimulation groups, the effect of rhythmic musical-electrical TNS was significantly stronger on rhythmic responses at the stimulated vs non-stimulated frequency ($t_{40} = 3.847$, $p = 4.2 \times 10^{-4}$, $d = 0.601$; data not shown). In sum, these neural results show that the rhythmic musical-electrical TNS facilitated DOC patients' rhythmic brain activity especially at the stimulation frequency.

3.3. Improved consciousness is related to enhanced rhythmic brain activity

To assess whether the observed enhancement of rhythmic brain activity was related to the patients' consciousness improvement, we tested for a positive correlation between the observed rhythmic response changes (gamma ASSR changes plus beta ASSR changes) and the observed CRS-R changes. For this analysis, we summed the gamma and beta ASSR changes and pooled across the stimulation groups to increase statistical power. The rationale for the pooling procedure is explained in the supplementary material. We found a weak significant positive correlation between the observed rhythmic response changes and the observed CRS-R changes from pretest to posttest (Spearman's $\rho = 0.252$, $p = 0.026$; Fig. 4), showing that stronger rhythmic brain-activity improvements may be accompanied by stronger consciousness improvements. This result suggests that improvements in consciousness are functionally coupled to increases in rhythmic brain activity.

3.4. No strong aftereffect of rhythmic stimulation on speech processing

In addition to rhythmic processes in beta and gamma range, our novel auditory speech paradigm allowed us to investigate speech processes as a potential source (or consequence) of patients' consciousness improvements. To this end, we extracted neural measures reflecting respectively the tracking of hierarchical linguistic structures and the detection of semantic violations, which were embedded in the auditory speech stimuli (see Methods). The results are shown in Fig. S2-3. In brief, we observed no systematic effect of rhythmic musical-electrical TNS on neural speech tracking and a facilitating effect of gamma, but not beta, stimulation on semantic violation detection. These side observations suggest that the observed effect of gamma stimulation on consciousness involved improvements in not only rhythmic but also semantic linguistic processes.

3.5. Responsiveness to rhythmic stimulation predicts one-year outcomes

The ordinal logistic regression analysis revealed that the five predictors explained 57.2 % of the variance in patients' one-year outcomes (Pseudo $R^2 = 0.572$, $p = 4.9 \times 10^{-5}$). The CRS-R total score at pretest and the response to rhythmic musical-electrical TNS (diagnostic improvement vs no such improvement) were significant predictors of a higher GOSE score (response to TNS: beta = 2.484, odds ratios (OR) = 11.989, $p = 0.005$; CRS-R: beta = 0.573, OR = 1.774, $p = 0.002$, Table 1). These results suggest that DOC patients' response to rhythmic musical-electrical TNS can predict their one-year outcomes.

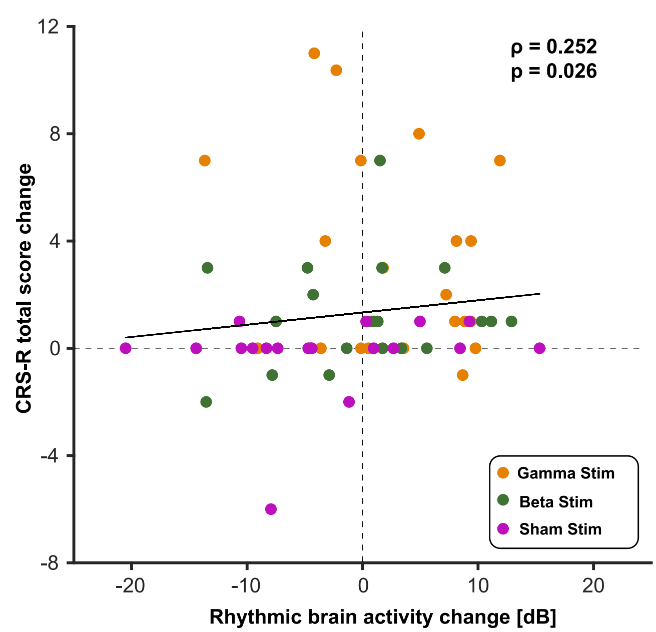

Fig. 4. Relation between changes in consciousness and changes in rhythmic brain activity in DOC patients. The scatterplot shows results from a correlation analysis testing for a functional coupling between changes in consciousness and changes in rhythmic brain activity. Correlation coefficient rho (ρ) and p-value describe, respectively, the strength and statistical significance of the coupling (linear regression line) across all patients. Orange, green, and magenta dots respectively represent patients in the gamma, beta, and sham-stimulation group.

Table 1
Predictive value of DOC patients' response to rhythmic musical-electrical TNS for one-year outcomes.

Predictor	Beta	OR	95 % CI		p-Value	Pseudo R^2	F	p-Value (model)
Age	-0.041	0.960	0.912	1.010	0.112	0.572	27.347	4.9×10^{-5}
Etiology/TBI	0.047	1.048	0.214	5.129	0.953			
Month since injury	0.234	1.264	0.970	1.644	0.083			
CRS-R total score	0.573	1.774	1.245	2.529	0.002			
Response to TNS/Yes	2.484	11.989	2.092	68.717	0.005			

OR: odds ratios, CI: confidence interval, TBI: traumatic brain injury.

4. Discussion

In this study, we found that rhythmic musical-electrical TNS leads to a significant improvement in DOC patients' consciousness and this improvement can predict their one-year outcomes. We further found that the stimulation leads to a significant increase in patients' rhythmic brain activity especially at the stimulation frequency. Moreover, the improvements in consciousness and rhythmic brain activity are significantly related to each other. Overall, our results provide strong evidence that non-invasive gamma/beta musical-electrical TNS can improve DOC patients' consciousness and propose frequency-specific enhancement of rhythmic brain activity as a potential neural mechanism.

4.1. Characteristics of the positive aftereffect of rhythmic musical-electrical TNS on patients' consciousness

We found that the musical-electrical TNS in gamma and beta range successfully improved DOC patients' consciousness. More specifically, 43.5 % of DOC patients showed an improvement of their diagnosis after being treated with gamma stimulation and 25 % after beta stimulation. These numbers are similar to, or higher than those, achieved with other non-invasive DOC treatments such as transcranial direct current stimulation (tDCS) (Hermann et al., 2020; Thibaut et al., 2014). It should be noted that patients in the present study and previous tDCS studies differed in their post-injury time. In the studies by Thibaut and colleagues (Thibaut et al., 2014), post-injury time varied from 18 days to 30 years (53 % of patients: longer than 12 months), whereas in our study it varied from 1 to 11 months. As DOC patients often recover 3–12 months after injury (Giacino et al., 2014; Giacino et al., 2018a), patients with a shorter post-injury time may have better prognosis. Thus, the earlier and narrower post-injury time range in our study may have facilitated the interpretation of our results, but it may have also increased our likelihood to observe a treatment effect. Note that our findings may not be attributed to spontaneous recovery or placebo effects, as we controlled for these potential confounds in the analysis.

We further observed that the rhythmic musical-electrical TNS impacted most strongly on our patients' visual and motor abilities, which is in line with previous studies using various types of non-invasive brain stimulation, such as tDCS (Martens et al., 2018; Thibaut et al., 2017), transcranial magnetic stimulation (Manganotti et al., 2013) and vagus-nerve stimulation (Noe et al., 2020). The strong effect on visual abilities may be explained by the fact that visual signs constitute a highly sensitive behavioral indicator of consciousness (Bagnato et al., 2017). The strong effect on patients' motor abilities may be due to the non-invasive electrical stimulation effects spreading to the motor cortex (Asamoah et al., 2019), which plays a major role in motor functions. We also observed an effect on patients' communication abilities specifically after gamma stimulation (see below).

Noteworthy, we observed that rhythmic musical-electrical TNS may be more effective for MCS patients than UWS patients (see supplementary material, section *Susceptibility of MCS and UWS patients' consciousness to rhythmic stimulation*), which is in line with other treatment studies reporting larger effects in MCS patients (Thibaut et al., 2014; Zhang et al., 2017). As mentioned in the results above, one potential explanation is that the present and previous samples were biased toward MCS patients. An alternative, perhaps more exciting potential explanation is that frontoparietal and thalamocortical connectivity tends to be more preserved in MCS than UWS (Laureys et al., 2004; Stender et al., 2014). This putatively stronger connectivity in MCS patients may have allowed the neural effects of electrical stimulation to spread more widely across the MCS patient's brain, which may have resulted in more widespread plasticity and stronger consciousness recovery (Naro et al., 2018).

4.2. Characteristics of the positive aftereffect of rhythmic musical-electrical TNS on patients' rhythmic brain activity

We found that musical-electrical TNS in beta range and especially gamma range leads to a significant frequency-specific enhancement of rhythmic brain activity. Remarkably, this observed frequency-specific neural enhancement outlasted the actual stimulation by at least 24 h.

Long-lasting (up to 70 min) frequency-specific enhancement of endogenous brain activity has been observed previously after tACS at alpha frequency (Kasten et al., 2016) and the duration of such aftereffects seems to be positively related to the duration of the stimulation (Nitsche et al., 2003; Vossen et al., 2015). Moreover, the strength of these aftereffects may be positively related to the strength of the instantaneous effects of the stimulation (Helfrich et al., 2014a; Helfrich et al., 2014b). Thus, the long-lasting neural aftereffect observed in our study may be related to our relatively long stimulation phase (which included 40 min of stimulation on each of five consecutive days) and probably strong instantaneous effects induced by the simultaneous application of relatively strong currents directly above the trigeminal nerve and potentially emotional musical stimuli carrying the same rhythm (Engineer et al., 2011; Marks et al., 2018; Martorell et al., 2019).

A candidate mechanism underlying the aftereffects of electrical brain stimulation is spike-timing dependent plasticity (Vossen et al., 2015). External rhythmic stimulation may "reshape" neuronal circuits by inducing adaptions in temporal patterns of synaptic activity (Feldman, 2012; Zaehle et al., 2010). These adaptations may persist and reverberate until after the stimulation, resulting in a long-lived rhythmic aftereffect (Caporale and Dan, 2008; Jones et al., 2020). Notably, the effect of external rhythmic stimulation on spike-timing-dependent plasticity depends on the similarity of the stimulation frequency and the resonance frequency of the neural circuit (Zaehle et al., 2010). Put differently, external rhythmic stimulation at a given frequency may have the strongest effect on neural circuits that have a matching resonance frequency. Based on these considerations, the frequency-specific aftereffects observed in our study may originate from an effect of the rhythmic musical-electrical TNS on spike-timing dependent plasticity in neural circuits with a similar resonance frequency as the stimulation.

We observed that the behavioral and neural effects of gamma stimulation (on patients' consciousness level, rhythmic brain activity, and semantic violation-detection ability) were descriptively stronger than those of beta stimulation, suggesting additional contributions from gamma stimulation to consciousness and brain activity. Our observations are in line with previous findings emphasizing a role of gamma activity over activity at other frequencies. For example, a study on lucid dreaming found a stronger effect of tACS at 40 Hz than 25 Hz (Voss et al., 2014). Another study observed a stronger correlation between consciousness and rhythmic brain activity at 40 Hz than at other frequencies

(Binder et al., 2017). Thus, stimulation at gamma frequency may contribute more strongly to consciousness than stimulation at other frequencies. However, it should be noted that frequency borders between oscillatory bands are defined rather broadly, so our beta frequency of 28 Hz may be considered as belonging to a lower gamma band as well (Voss et al., 2014).

4.3. Neural basis of the positive aftereffect of rhythmic musical-electrical TNS on patients' consciousness

Considering rhythmic musical-electrical TNS as a potential clinical treatment, it is important to understand the mechanism by which it improves consciousness. We found that the patients' consciousness improvement was significantly positively related to the rhythmic brain activity increase; however, a small subset of patients showed the opposite pattern, implying that generalization of our results to the population should be done with some caution. With the cautionary remark that this correlation was of rather modest strength, the observed positive brain-behavior link may be interpreted in two alternative ways. Firstly, the stimulation might have improved consciousness, which consequently enhanced rhythmic brain activity. However, this interpretation may be difficult to reconcile with our observation that the stimulation effect on rhythmic brain activity is frequency-specific, rendering this alternative less plausible.

A second, perhaps more exciting interpretation is that the stimulation directly enhanced rhythmic brain activity, which consequently improved consciousness. This interpretation can be reconciled more easily with the observed frequency-specific neural effect and is in line with ideas from a previous non-invasive human brain-stimulation study showing a similar pattern of behavioral and neural effects. In that study, a positive frequency-specific effect of 25 Hz and 40 Hz tACS on both frontotemporal cortical activity (assessed with online EEG) and lucid dreaming (assessed with a validated scale after sleep) was found and interpreted as a causal role of frequency-specific rhythmic brain activity for consciousness (Voss et al., 2014). As mentioned in the Introduction, direct electrical stimulation of the thalamus may improve consciousness (Rezaei Haddad et al., 2019; Schiff, 2008; Schiff et al., 2007) and our rhythmic musical-electrical TNS was designed to induce strong synchronous activity in multisensory thalamic nuclei and the cortex; thus the observed consciousness improvement possibly originated in patients' thalamus and/or its interaction with hierarchically higher structures in cortex. To further disentangle direct from indirect effects of a given brain structure on consciousness would require experimentally manipulating activity in that brain structure while keeping all other brain activities constant, which would be a difficult endeavor.

4.4. Potential link between gamma activity and patients' speech processing abilities

Our results indicate some improvements in DOC patients' ability to process speech after receiving gamma stimulation, as reflected by significant increases in both patients' communication ability (as assessed with the CRS-R communication subscale) and their semantic violation-detection ability (as assessed with event-related potentials to semantically incongruent words). Communication disability in neuropsychiatric disorders (e.g., Autism spectrum disorders) (Rojas et al., 2011) has been linked to abnormal gamma activity. Similarly, studies using gamma tACS have shown that certain aspects of speech perception (e.g., formant integration and phonetic categorization) may depend on gamma activity (Giraud and Poeppel, 2012; Preisig et al., 2020). Moreover, detection of changes in repeated utterances has been shown to lead to increased gamma activity (Basirat et al., 2008). Based on these studies, it is possible that our observation of a positive effect of gamma stimulation on speech processing resulted from the observed enhancement of gamma activity.

We found no systematic effect of gamma stimulation on neural speech tracking. Our observation of a significant effect of gamma stimulation on semantic violation detection, but not speech tracking, may indicate that these two processes operate at different levels of auditory speech analysis. Detection of semantic violations (as assessed by the N400) has been observed during sleep, although with lower strength than during wakefulness (Ibanez et al., 2006). Contrarily, neural tracking of sentential structure is not observable during sleep (Makov et al., 2017). This suggests that detection of semantic deviations reflects an automatic process (Ibanez et al., 2006; Kiefer, 2002), whereas tracking of sentential structure relies on continuous comprehension using top-down lexical knowledge (Ding et al., 2018). Therefore, it is possible that our gamma stimulation affected only low levels of speech processing (possibly through an increase in gamma activity; see above), while leaving higher-order speech processes involved in continuous speech comprehension unaffected.

4.5. Patients' response to rhythmic musical-electrical TNS predicts one-year outcomes

While the prognostic value of CRS-R score has been established in previous work (Hamilton et al., 2020; Lucca et al., 2019), our finding of an additional prognostic value of patients' response to the rhythmic musical-electrical TNS is novel. This predictive value might be attributed to long-lasting effects of the stimulation. More specifically, the rhythmic musical-electrical TNS may have restored patients' oscillatory brain activity and consciousness, and patients who received this benefit possibly could preserve the elevated consciousness level, leading to a more successful recovery-one year later. Another possible interpretation relates to the preservation of intact sensory neural pathways, which is used to aid medical prognostication after injury (Carter and Butt, 2001; Edlow et al., 2021). The patients who benefitted from the stimulation might have preserved the integrity of their sensory neural pathways to transmit auditory and tactile sensations up to the cerebral cortex and, consequently, these patients showed a more successful long-term recovery.

4.6. Potential limitations

However, our interpretation requires a few cautionary remarks. First, it remains unclear whether the effects of musical-electrical TNS are driven by the music, the electrical stimulation, and/or their interplay. Future research may disambiguate the relative effectiveness of the different stimulation modalities by applying them also in isolation. Second, although we aimed to match the three patient groups for potentially confounding variables (e.g., time since injury), the present sample did not allow us to do so perfectly. Despite this imperfection, these variables unlikely constituted a confound in our study as none of them varied systemically across the groups. Perfect matching can be accomplished more easily with a within-subject (crossover) design; however, this type of design induces the risk of confounding order effects or carryover effects between consecutive treatments. Third, the patient blinding for acoustic stimulation was not optimal, as patients in the sham group might have been able to notice the muting of the stimulation. However, this unlikely induced a systematic bias as patients kept on all transducers throughout the treatment and were kept unaware of (any difference between) the stimulation conditions. Finally, the use of patient exclusion criteria implies that the findings may not generalize to the entire population of DOC patients, specifically those with major skull-bone defects or major brain-tissue defects.

5. Conclusion

The rhythmic musical-electrical TNS provides a neuroscience-informed, patient-friendly approach to improve consciousness and predict one-year outcomes in DOC patients, indicating its high potential for clinical use. The benefit seems to be facilitated by a frequency-specific enhancement of rhythmic neural processing.

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CRediT authorship contribution statement

Min Wu: Conceptualization, Investigation, Formal analysis, Data curation, Methodology, Software, Funding acquisition, Writing – original draft, Writing – review & editing. **Benyan Luo:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing. **Yamei Yu:** Data curation, Writing – review & editing. **Xiaoxia Li:** Data curation, Writing – review & editing. **Jian Gao:** Methodology, Writing – review & editing. **Jingqi Li:** Resources, Data curation, Writing – review & editing. **Bettina Sorger:** Conceptualization, Supervision, Writing – review & editing. **Lars Riecke:** Conceptualization, Methodology, Resources, Data curation, Supervision, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nicl.2022.103170.

References

Adair, D., Truong, D., Esmaeilpour, Z., Gebodh, N., Borges, H., Ho, L., Bremner, J.D., Badran, B.W., Napadow, V., Clark, V.P., Bikson, M., 2020. Electrical stimulation of cranial nerves in cognition and disease. Brain Stimulation 13, 717–750. https://doi.org/10.1016/j.brs.2020.02.019.

Antal, A., Paulus, W., 2013. Transcranial alternating current stimulation (tACS). Front. Hum. Neurosci. 7, 317. https://doi.org/10.3389/fnhum.2013.00317.

Asamoah, B., Khatoun, A., Mc Laughlin, M., 2019. tACS motor system effects can be caused by transcutaneous stimulation of peripheral nerves. Nat. Commun. 10, 266. https://doi.org/10.1038/s41467-018-08183-w.

Avins, A.L., Cherkin, D.C., Sherman, K.J., Goldberg, H., Pressman, A., 2012. Should we reconsider the routine use of placebo controls in clinical research? Trials 13, 44. https://doi.org/10.1186/1745-6215-13-44.

Bagnato, S., Boccagni, C., Sant'Angelo, A., Fingelkurts, A.A., Fingelkurts, A.A., Galardi, G., 2017. Longitudinal Assessment of Clinical Signs of Recovery in Patients with Unresponsive Wakefulness Syndrome after Traumatic or Nontraumatic Brain Injury. J. Neurotrauma 34, 535–539. https://doi.org/10.1089/neu.2016.4418.

Basirat, A., Sato, M., Schwartz, J.L., Kahane, P., Lachaux, J.P., 2008. Parieto-frontal gamma band activity during the perceptual emergence of speech forms. Neuroimage 42, 404–413. https://doi.org/10.1016/j.neuroimage.2008.03.063.

Belouchrani, A., AbedMeraim, K., Cardoso, J.F., Moulines, E., 1997. A blind source separation technique using second-order statistics. IEEE Trans. Signal Process. 45, 434–444.

Binder, M., Gorska, U., Griskova-Bulanova, I., 2017. 40Hz auditory steady-state responses in patients with disorders of consciousness: Correlation between phase-locking index and Coma Recovery Scale-Revised score. Clin. Neurophysiol. 128, 799–806. https://doi.org/10.1016/j.clinph.2017.02.012.

Boucher, P.O., Ozdemir, R.A., Momi, D., Burke, M.J., Jannati, A., Fried, P.J., Pascual-Leone, A., Shafi, M.M., Santarnecchi, E., 2021. Sham-derived effects and the minimal reliability of theta burst stimulation. Sci. Rep. 11, 21170. https://doi.org/10.1038/s41598-021-98751-w.

Caporale, N., Dan, Y., 2008. Spike timing-dependent plasticity: a Hebbian learning rule. Annu. Rev. Neurosci. 31, 25–46. https://doi.org/10.1146/annurev.neuro.31.060407.125639.

Carter, B.G., Butt, W., 2001. Review of the use of somatosensory evoked potentials in the prediction of outcome after severe brain injury. Crit. Care Med. 29, 178–186. https://doi.org/10.1097/00003246-200101000-00036.

Cavinato, M., Genna, C., Manganotti, P., Formaggio, E., Storti, S.F., Campostrini, S., Arcaro, C., Casanova, E., Petrone, V., Piperno, R., Piccione, F., 2015. Coherence and Consciousness: Study of Fronto-Parietal Gamma Synchrony in Patients with Disorders of Consciousness. Brain Topogr. 28, 570–579. https://doi.org/10.1007/s10548-014-0383-5.

Clements-Cortes, A., Ahonen, H., Evans, M., Freedman, M., Bartel, L., Acquaah-Mensah, G., 2016. Short-Term Effects of Rhythmic Sensory Stimulation in Alzheimer's Disease: An Exploratory Pilot Study. J. Alzheimers Dis. 52 (2), 651–660.

Conlon, B., Langguth, B., Hamilton, C., Hughes, S., Meade, E., Connor, C.O., Schecklmann, M., Hall, D.A., Vanneste, S., Leong, S.L., Subramaniam, T., D'Arcy, S., Lim, H.H., 2020. Bimodal neuromodulation combining sound and tongue stimulation reduces tinnitus symptoms in a large randomized clinical study. Sci. Transl. Med. 12 https://doi.org/10.1126/scitranslmed.abb2830.

DeGiorgio, C.M., Soss, J., Cook, I.A., Markovic, D., Gornbein, J., Murray, D., Oviedo, S., Gordon, S., Corralle-Leyva, G., Kealey, C.P., Heck, C.N., 2013. Randomized controlled trial of trigeminal nerve stimulation for drug-resistant epilepsy. Neurology 80, 786–791. https://doi.org/10.1212/WNL.0b013e318285c11a.

Delorme, A., Makeig, S., 2004. EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. J. Neurosci. Methods 134 (1), 9–21.

Ding, N., Melloni, L., Zhang, H., Tian, X., Poeppel, D., 2016. Cortical tracking of hierarchical linguistic structures in connected speech. Nat. Neurosci. 19, 158–164. https://doi.org/10.1038/nn.4186.

Ding, N., Pan, X., Luo, C., Su, N., Zhang, W., Zhang, J., 2018. Attention Is Required for Knowledge-Based Sequential Grouping: Insights from the Integration of Syllables into Words. J. Neurosci. 38, 1178–1188. https://doi.org/10.1523/JNEUROSCI.2606-17.2017.

Edlow, B.L., Claassen, J., Schiff, N.D., Greer, D.M., 2021. Recovery from disorders of consciousness: mechanisms, prognosis and emerging therapies. Nature Reviews Neurology 17, 135–156. https://doi.org/10.1038/s41582-020-00428-x.

Engineer, N.D., Riley, J.R., Seale, J.D., Vrana, W.A., Shetake, J.A., Sudanagunta, S.P., Borland, M.S., Kilgard, M.P., 2011. Reversing pathological neural activity using targeted plasticity. Nature 470, 101–104. https://doi.org/10.1038/nature09656.

Fan, S., Wu, X., Xie, M., Li, X., Liu, C., Su, Y., Chen, Y., Wu, S., Ma, C., 2019. Trigeminal nerve stimulation successfully awakened an unconscious patient. Brain Stimulation 12, 361–363. https://doi.org/10.1016/j.brs.2018.11.001.

Feldman, D.E., 2012. The spike-timing dependence of plasticity. Neuron 75, 556–571. https://doi.org/10.1016/j.neuron.2012.08.001.

Flores, F.J., Hartnack, K.E., Fath, A.B., Kim, S.E., Wilson, M.A., Brown, E.N., Purdon, P. L., 2017. Thalamocortical synchronization during induction and emergence from propofol-induced unconsciousness. Proc Natl Acad Sci U S A 114, E6660–E6668. https://doi.org/10.1073/pnas.1700148114.

Gaillard, R., Dehaene, S., Adam, C., Clémenceau, S., Hasboun, D., Baulac, M., Cohen, L., Naccache, L., Ungerleider, L., 2009. Converging intracranial markers of conscious access. PLoS Biol. 7 (3), e1000061.

Giacino, J.T., Kalmar, K., Whyte, J., 2004. The JFK Coma Recovery Scale-Revised: measurement characteristics and diagnostic utility. Arch. Phys. Med. Rehabil. 85, 2020–2029. https://doi.org/10.1016/j.apmr.2004.02.033.

Giacino, J.T., Fins, J.J., Laureys, S., Schiff, N.D., 2014. Disorders of consciousness after acquired brain injury: the state of the science. Nature Reviews Neurology 10, 99–114. https://doi.org/10.1038/nrneurol.2013.279.

Giacino, J.T., Katz, D.I., Schiff, N.D., Whyte, J., Ashman, E.J., Ashwal, S., Barbano, R., Hammond, F.M., Laureys, S., Ling, G.S.F., Nakase-Richardson, R., Seel, R.T., Yablon, S., Getchius, T.S.D., Gronseth, G.S., Armstrong, M.J., 2018a. Comprehensive systematic review update summary: Disorders of consciousness: Report of the Guideline Development, Dissemination, and Implementation Subcommittee of the American Academy of Neurology; the American Congress of Rehabilitation Medicine; and the National Institute on Disability, Independent Living, and Rehabilitation Research. Neurology 91, 461–470. https://doi.org/10.1212/WNL.0000000000005928.

Giacino, J.T., Katz, D.I., Schiff, N.D., Whyte, J., Ashman, E.J., Ashwal, S., Barbano, R., Hammond, F.M., Laureys, S., Ling, G.S.F., Nakase-Richardson, R., Seel, R.T., Yablon, S., Getchius, T.S.D., Gronseth, G.S., Armstrong, M.J., 2018b. Practice guideline update recommendations summary: Disorders of consciousness: Report of the Guideline Development, Dissemination, and Implementation Subcommittee of the American Academy of Neurology; the American Congress of Rehabilitation Medicine; and the National Institute on Disability, Independent Living, and Rehabilitation Research. Neurology 91, 450–460. https://doi.org/10.1212/WNL.0000000000005926.

Giraud, A.L., Poeppel, D., 2012. Cortical oscillations and speech processing: emerging computational principles and operations. Nat. Neurosci. 15, 511–517. https://doi.org/10.1038/nn.3063.

Gorgulho, A.A., Fernandes, F., Damiani, L.P., Barbosa, D.A.N., Cury, A., Lasagno, C.M., Bueno, P.R.T., Santos, B.F.O., Santos, R.H.N., Berwanger, O., Cavalcanti, A.B., Teixeira, M.J., Moreno, R.A., De Salles, A.A.F., 2019. Double Blinded Randomized Trial of Subcutaneous Trigeminal Nerve Stimulation as Adjuvant Treatment for Major Unipolar Depressive Disorder. Neurosurgery 85, 717–728. https://doi.org/10.1093/neuros/nyy420.

Gorska, U., Binder, M., 2019. Low- and medium-rate auditory steady-state responses in patients with prolonged disorders of consciousness correlate with Coma Recovery Scale - Revised score. Int. J. Psychophysiol. 144, 56–62. https://doi.org/10.1016/j.ijpsycho.2019.08.001.

Gui, P., Jiang, Y., Zang, D., Qi, Z., Tan, J., Tanigawa, H., Jiang, J., Wen, Y., Xu, L., Zhao, J., Mao, Y., Poo, M.M., Ding, N., Dehaene, S., Wu, X., Wang, L., 2020. Assessing the depth of language processing in patients with disorders of consciousness. Nat. Neurosci. 23, 761–770. https://doi.org/10.1038/s41593-020-0639-1.

Hamilton, J.A., Perrin, P.B., Campbell, T.A., Danish, S.J., Goldstein, A.L., 2020. Predicting emergence from a disorder of consciousness using the Coma Recovery Scale-Revised. Neuropsychol Rehabil 30, 266–280. https://doi.org/10.1080/09602011.2018.1461656.

Helfrich, R.F., Knepper, H., Nolte, G., Strüber, D., Rach, S., Herrmann, C.S., Schneider, T.R., Engel, A.K., Jensen, O., 2014a. Selective modulation of interhemispheric functional connectivity by HD-tACS shapes perception. PLoS Biol. 12 (12), e1002031.

Helfrich, R.F., Schneider, T.R., Rach, S., Trautmann-Lengsfeld, S.A., Engel, A.K., Herrmann, C.S., 2014b. Entrainment of brain oscillations by transcranial alternating current stimulation. Curr. Biol. 24, 333–339. https://doi.org/10.1016/j.cub.2013.12.041.

Hermann, B., Raimondo, F., Hirsch, L., Huang, Y.u., Denis-Valente, M., Pérez, P., Engemann, D., Faugeras, F., Weiss, N., Demeret, S., Rohaut, B., Parra, L.C., Sitt, J.D., Naccache, L., 2020. Combined behavioral and electrophysiological evidence for a direct cortical effect of prefrontal tDCS on disorders of consciousness. Sci. Rep. 10 (1).

Iaccarino, H.F., Singer, A.C., Martorell, A.J., Rudenko, A., Gao, F., Gillingham, T.Z., Mathys, H., Seo, J., Kritskiy, O., Abdurrob, F., Adaikkan, C., Canter, R.G., Rueda, R., Brown, E.N., Boyden, E.S., Tsai, L.H., 2016. Gamma frequency entrainment attenuates amyloid load and modifies microglia. Nature 540, 230–235. https://doi.org/10.1038/nature20587.

Ibanez, A., Lopez, V., Cornejo, C., 2006. ERPs and contextual semantic discrimination: degrees of congruence in wakefulness and sleep. Brain Lang. 98, 264–275. https://doi.org/10.1016/j.bandl.2006.05.005.

Jaeger, M., Bleichner, M.G., Bauer, A.R., Mirkovic, B., Debener, S., 2018. Did You Listen to the Beat? Auditory Steady-State Responses in the Human Electroencephalogram at 4 and 7 Hz Modulation Rates Reflect Selective Attention. Brain Topogr. 31, 811–826. https://doi.org/10.1007/s10548-018-0637-8.

Jones, K.T., Johnson, E.L., Tauxe, Z.S., Rojas, D.C., 2020. Modulation of auditory gamma-band responses using transcranial electrical stimulation. J. Neurophysiol. 123, 2504–2514. https://doi.org/10.1152/jn.00003.2020.

Kallionpaa, R.E., Scheinin, A., Kallionpaa, R.A., Sandman, N., Kallioinen, M., Laitio, R., Laitio, T., Kaskinoro, K., Kuusela, T., Revonsuo, A., Scheinin, H., Valli, K., 2018. Spoken words are processed during dexmedetomidine-induced unresponsiveness. Br. J. Anaesth. 121, 270–280. https://doi.org/10.1016/j.bja.2018.04.032.

Kasten, F.H., Dowsett, J., Herrmann, C.S., 2016. Sustained Aftereffect of alpha-tACS Lasts Up to 70 min after Stimulation. Front. Hum. Neurosci. 10, 245. https://doi.org/10.3389/fnhum.2016.00245.

Kasten, F.H., Herrmann, C.S., 2017. Transcranial Alternating Current Stimulation (tACS) Enhances Mental Rotation Performance during and after Stimulation. Front. Hum. Neurosci. 11, 2. https://doi.org/10.3389/fnhum.2017.00002.

Kiefer, M., 2002. The N400 is modulated by unconsciously perceived masked words: further evidence for an automatic spreading activation account of N400 priming effects. Cognitive Brain Research 13 (1), 27–39.

Koch, C., Massimini, M., Boly, M., Tononi, G., 2016. Neural correlates of consciousness: progress and problems. Nat. Rev. Neurosci. 17, 307–321. https://doi.org/10.1038/nrn.2016.22.

Kondziella, D., Bender, A., Diserens, K., van Erp, W., Estraneo, A., Formisano, R., Laureys, S., Naccache, L., Ozturk, S., Rohaut, B., Sitt, J.D., Stender, J., Tiainen, M., Rossetti, A.O., Gosseries, O., Chatelle, C., 2020. European Academy of Neurology guideline on the diagnosis of coma and other disorders of consciousness. Eur. J. Neurol. 27 (5), 741–756.

Laureys, S., Owen, A.M., Schiff, N.D., 2004. Brain function in coma, vegetative state, and related disorders. Lancet Neurol. 3, 537–546. https://doi.org/10.1016/S1474-4422(04)00852-X.

Lucca, L.F., Lofaro, D., Pignolo, L., Leto, E., Ursino, M., Cortese, M.D., Conforti, D., Tonin, P., Cerasa, A., 2019. Outcome prediction in disorders of consciousness: the role of coma recovery scale revised. BMC Neurology 19, 68. https://doi.org/10.1186/s12883-019-1293-7.

Makov, S., Sharon, O., Ding, N., Ben-Shachar, M., Nir, Y., Zion Golumbic, E., 2017. Sleep Disrupts High-Level Speech Parsing Despite Significant Basic Auditory Processing. J. Neurosci. 37, 7772–7781. https://doi.org/10.1523/JNEUROSCI.0168-17.2017.

Manganotti, P., Formaggio, E., Storti, S.F., Fiaschi, A., Battistin, L., Tonin, P., Piccione, F., Cavinato, M., 2013. Effect of high-frequency repetitive transcranial magnetic stimulation on brain excitability in severely brain-injured patients in minimally conscious or vegetative state. Brain Stimulation 6, 913–921. https://doi.org/10.1016/j.brs.2013.06.006.

Maris, E., Oostenveld, R., 2007. Nonparametric statistical testing of EEG- and MEG-data. J. Neurosci. Methods 164, 177–190. https://doi.org/10.1016/j.jneumeth.2007.03.024.

Marks, K.L., Martel, D.T., Wu, C., Basura, G.J., Roberts, L.E., Schvartz-Leyzac, K.C., Shore, S.E., 2018. Auditory-somatosensory bimodal stimulation desynchronizes brain circuitry to reduce tinnitus in guinea pigs and humans. Sci. Transl. Med. 10 https://doi.org/10.1126/scitranslmed.aal3175.

Martens, G., Lejeune, N., O'Brien, A.T., Fregni, F., Martial, C., Wannez, S., Laureys, S., Thibaut, A., 2018. Randomized controlled trial of home-based 4-week tDCS in chronic minimally conscious state. Brain Stimulation 11, 982–990. https://doi.org/10.1016/j.brs.2018.04.021.

Martorell, A.J., Paulson, A.L., Suk, H.-J., Abdurrob, F., Drummond, G.T., Guan, W., Young, J.Z., Kim, D.-W., Kritskiy, O., Barker, S.J., Mangena, V., Prince, S.M., Brown, E.N., Chung, K., Boyden, E.S., Singer, A.C., Tsai, L.-H., 2019. Multi-sensory Gamma Stimulation Ameliorates Alzheimer's-Associated Pathology and Improves Cognition. Cell 177 (2), 256–271.e22.

McCormick, D.A., Bal, T., 1994. Sensory gating mechanisms of the thalamus. Curr. Opin. Neurobiol. 4, 550–556. https://doi.org/10.1016/0959-4388(94)90056-6.

Melloni, L., Molina, C., Pena, M., Torres, D., Singer, W., Rodriguez, E., 2007. Synchronization of neural activity across cortical areas correlates with conscious perception. J. Neurosci. 27, 2858–2865. https://doi.org/10.1523/JNEUROSCI.4623-06.2007.

Naro, A., Bramanti, P., Leo, A., Russo, M., Calabro, R.S., 2016. Transcranial Alternating Current Stimulation in Patients with Chronic Disorder of Consciousness: A Possible Way to Cut the Diagnostic Gordian Knot? Brain Topogr. 29, 623–644. https://doi.org/10.1007/s10548-016-0489-z.

Naro, A., Bramanti, A., Leo, A., Bramanti, P., Calabro, R.S., 2018. Metaplasticity: A Promising Tool to Disentangle Chronic Disorders of Consciousness Differential Diagnosis. Int. J. Neural Syst. 28, 1750059. https://doi.org/10.1142/S0129065717500599.

Nitsche, M.A., Nitsche, M.S., Klein, C.C., Tergau, F., Rothwell, J.C., Paulus, W., 2003. Level of action of cathodal DC polarisation induced inhibition of the human motor cortex. Clin. Neurophysiol. 114, 600–604. https://doi.org/10.1016/s1388-2457(02)00412-1.

Noe, E., Ferri, J., Colomer, C., Moliner, B., O'Valle, M., Ugart, P., Rodriguez, C., Llorens, R., 2020. Feasibility, safety and efficacy of transauricular vagus nerve stimulation in a cohort of patients with disorders of consciousness. Brain Stimulation 13, 427–429. https://doi.org/10.1016/j.brs.2019.12.005.

Pastor, M.A., Artieda, J., Arbizu, J., Marti-Climent, J.M., Peñuelas, I., Masdeu, J.C., 2002. Activation of human cerebral and cerebellar cortex by auditory stimulation at 40 Hz. J. Neurosci. 22 (23), 10501–10506.

Pion-Tonachini, L., Kreutz-Delgado, K., Makeig, S., 2019. ICLabel: An automated electroencephalographic independent component classifier, dataset, and website. Neuroimage 198, 181–197. https://doi.org/10.1016/j.neuroimage.2019.05.026.

Preisig, B.C., Sjerps, M.J., Hervais-Adelman, A., Kosem, A., Hagoort, P., Riecke, L., 2020. Bilateral Gamma/Delta Transcranial Alternating Current Stimulation Affects Interhemispheric Speech Sound Integration. J Cogn Neurosci 32, 1242–1250. https://doi.org/10.1162/jocn_a_01498.

Redinbaugh, M.J., Phillips, J.M., Kambi, N.A., Mohanta, S., Andryk, S., Dooley, G.L., Afrasiabi, M., Raz, A., Saalmann, Y.B., 2020. Thalamus Modulates Consciousness via Layer-Specific Control of Cortex. Neuron 106 (1), 66–75.e12.

Reuter, U., McClure, C., Liebler, E., Pozo-Rosich, P., 2019. Non-invasive neuromodulation for migraine and cluster headache: a systematic review of clinical trials. J. Neurol. Neurosurg. Psychiatry 90, 796–804. https://doi.org/10.1136/jnnp-2018-320113.

Rezaei Haddad, A., Lythe, V., Green, A.L., 2019. Deep Brain Stimulation for Recovery of Consciousness in Minimally Conscious Patients After Traumatic Brain Injury: A Systematic Review. Neuromodulation 22, 373–379. https://doi.org/10.1111/ner.12944.

Rojas, D.C., Teale, P.D., Maharajh, K., Kronberg, E., Youngpeter, K., Wilson, L.B., Wallace, A., Hepburn, S., 2011. Transient and steady-state auditory gamma-band responses in first-degree relatives of people with autism spectrum disorder. Mol Autism 2, 11. https://doi.org/10.1186/2040-2392-2-11.

Schiff, N.D., 2008. Central thalamic contributions to arousal regulation and neurological disorders of consciousness. Ann. N. Y. Acad. Sci. 1129, 105–118. https://doi.org/10.1196/annals.1417.029.

Schiff, N.D., 2010. Recovery of consciousness after brain injury: a mesocircuit hypothesis. Trends Neurosci. 33, 1–9. https://doi.org/10.1016/j.tins.2009.11.002.

Schiff, N.D., Giacino, J.T., Kalmar, K., Victor, J.D., Baker, K., Gerber, M., Fritz, B., Eisenberg, B., Biondi, T., O'Connor, J., Kobylarz, E.J., Farris, S., Machado, A., McCagg, C., Plum, F., Fins, J.J., Rezai, A.R., 2007. Behavioural improvements with thalamic stimulation after severe traumatic brain injury. Nature 448, 600–603. https://doi.org/10.1038/nature06041.

Sherman, S.M., Guillery, R.W., 1996. Functional organization of thalamocortical relays. J. Neurophysiol. 76, 1367–1395. https://doi.org/10.1152/jn.1996.76.3.1367.

Steinmann, S., Leicht, G., Ertl, M., Andreou, C., Polomac, N., Westerhausen, R., Friederici, A.D., Mulert, C., 2014. Conscious auditory perception related to long-range synchrony of gamma oscillations. Neuroimage 100, 435–443. https://doi.org/10.1016/j.neuroimage.2014.06.012.

Stender, J., Gosseries, O., Bruno, M.-A., Charland-Verville, V., Vanhaudenhuyse, A., Demertzi, A., Chatelle, C., Thonnard, M., Thibaut, A., Heine, L., Soddu, A., Boly, M., Schnakers, C., Gjedde, A., Laureys, S., 2014. Diagnostic precision of PET imaging and functional MRI in disorders of consciousness: a clinical validation study. The Lancet 384, 514–522. https://doi.org/10.1016/s0140-6736(14)60042-8.

Supp, G.G., Siegel, M., Hipp, J.F., Engel, A.K., 2011. Cortical hypersynchrony predicts breakdown of sensory processing during loss of consciousness. Curr. Biol. 21, 1988–1993. https://doi.org/10.1016/j.cub.2011.10.017.

Thibaut, A., Bruno, M.A., Ledoux, D., Demertzi, A., Laureys, S., 2014. tDCS in patients with disorders of consciousness: sham-controlled randomized double-blind study. Neurology 82, 1112–1118. https://doi.org/10.1212/WNL.0000000000000260.

Thibaut, A., Wannez, S., Donneau, A.F., Chatelle, C., Gosseries, O., Bruno, M.A., Laureys, S., 2017. Controlled clinical trial of repeated prefrontal tDCS in patients with chronic minimally conscious state. Brain Inj. 31, 466–474. https://doi.org/10.1080/02699052.2016.1274776.

Voss, U., Holzmann, R., Hobson, A., Paulus, W., Koppehele-Gossel, J., Klimke, A., Nitsche, M.A., 2014. Induction of self awareness in dreams through frontal low current stimulation of gamma activity. Nat. Neurosci. 17, 810–812. https://doi.org/10.1038/nn.3719.

Vossen, A., Gross, J., Thut, G., 2015. Alpha Power Increase After Transcranial Alternating Current Stimulation at Alpha Frequency (alpha-tACS) Reflects Plastic Changes Rather Than Entrainment. Brain Stimul 8, 499–508. https://doi.org/10.1016/j.brs.2014.12.004.

Wannez, S., Heine, L., Thonnard, M., Gosseries, O., Laureys, S., 2017. The repetition of behavioral assessments in diagnosis of disorders of consciousness. Ann. Neurol. 81 (6), 883–889.

Wilson, L., Pettigrew, L., Teasdale, G., 1998. Structured Interviews for the Glasgow Outcome Scale and the Extended Glasgow Outcome Scale: Guidelines for Their Use. J. Neurotrauma 15, 573–585. https://doi.org/10.1089/neu.1998.15.573.

Zaehle, T., Rach, S., Herrmann, C.S., Aleman, A., 2010. Transcranial alternating current stimulation enhances individual alpha activity in human EEG. PLoS ONE 5 (11), e13766.

Zhang, Y., Song, W., Du, J., Huo, S., Shan, G., Li, R., 2017. Transcranial Direct Current Stimulation in Patients with Prolonged Disorders of Consciousness: Combined Behavioral and Event-Related Potential Evidence. Front. Neurol. 8, 620. https://doi.org/10.3389/fneur.2017.00620.