Non-Invasive Rhythmic Musical-Electric Trigeminal Nerve Stimulation Improves Consciousness in Patients with Disorders of Consciousness

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Abstract: Disorders of consciousness (DOC) are often accompanied by aberrant oscillatory neural activity in the thalamus and cerebral cortex. Patient-friendly non-invasive treatments targeting this functional anomaly are still missing. We propose and validate a novel approach that aims to restore DOC patients' thalamocortical oscillations by combining rhythmic trigeminal-nerve stimulation (TNS) with comodulated musical stimulation. In a cluster-randomized, placebo-controlled, double-blinded, pretest-posttest clinical study, we show that application of this multisensory approach for 40 min on five consecutive days reliably leads to long-lasting improvements in DOC patients' consciousness (assessed with Coma Recovery Scale-Revised) and oscillatory brain activity at the musical-electric TNS frequency (assessed with electroencephalography and a novel rhythmic auditory-speech paradigm). We found diagnostic improvement in 47% of patients in minimally conscious state and a positive relationship between patients' behavioral and neural improvements. Based on this evidence we argue that non-invasive musical-electric TNS may serve as an effective patient-friendly DOC treatment and suggest frequency-specific oscillatory neural enhancement as its mode of action.

Introduction

Severe brain injury often disrupts arousal and awareness systems, resulting in disorders of consciousness (DOC). With progress in intensive care, the number of hospitalized patients suffering from DOC has increased, leading to enormous costs for long-term care and immense burden for caretakers and society. In recent decades, a growing body of treatments has been developed with an increasing number of successfully treated cases. Besides pharmacological interventions (e.g., Zolpidem), non-invasive sensory and electric stimulation have shown promising result[1-3]. Despite these efforts, the number of compelling randomized controlled trials is still very limited and only few treatment guidelines for DOC patients exist to date.

A plausible approach to alleviate behavioural symptoms of neurological disease is to treat the specific brain functions impaired by the disease[4-6]. In the case of DOC, several findings hint to a fundamental role of neural oscillations in thalamic and cortical structures[7,8]. For example, injection of propofol has been shown to induce both an unconscious state and a perturbation in neural oscillations in the thalamocortical system[9-11]. Similarly, invasive application of electric stimulation to the thalamus (deep-brain stimulation, DBS) has been shown to improve consciousness in DOC patients[12-14]. Moreover, DOC patients' consciousness level has been shown to correlate positively with the strength of oscillatory auditory steady-state responses (ASSR, sustained phase-locked responses to rhythmic auditory stimulation) in the cerebral cortex specifically at gamma (~40Hz) frequency[15,16]. Thus, abnormal states of consciousness may arise from an anomaly within the thalamocortical oscillatory system, in particular reduced cortical gamma oscillations. Despite the evidence for abnormal thalamocortical oscillations in DOC patients, their causal role for

consciousness has not been fully determined, leaving their therapeutic value unclear.

Compared to DBS, a safer and more convenient method to potentially restore neural oscillations is transcranial alternating current stimulation (tACS)[17,18]. tACS involves the non-invasive application of an alternating current through electrodes at the scalp, which is thought to entrain cortical oscillations under these electrodes. The neural effectiveness of tACS is currently debated based on findings showing that only little electric current reaches the cortex directly through the skull (due to the relatively high conductivity of the scalp) and cortical effects may be an epiphenomenon of co-stimulation of peripheral nerves[19]. Based on these considerations, a more effective non-invasive approach to restore neural oscillations may be transcutaneous stimulation[20], which involves the application of an electric current to the skin directly above a peripheral or cranial nerve. A promising target for transcutaneous stimulation in DOC patients may be the trigeminal nerve, which is the largest cranial nerve. This nerve is critical for socially relevant somatosensory-motor functions of the face and has abundant anatomical projections to somatosensory nuclei of the thalamus in the midbrain[21,22], 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[23], depressive disorder[24] and migraine[25]. For DOC, so far only a single study in a single patient has been published, with promising results[26]. A systematic investigation of the potential of TNS for DOC treatment is still missing.

Besides electric stimulation, neural oscillations may be restored with sensory stimulation. Application of rhythmic auditory or visual stimulation induces

modulations in neural oscillations specifically at the frequency of the stimulation[27-29]. Thus, rhythmic sensory stimulation at gamma frequency may be exploited to alleviate cognitive dysfunctions accompanied by abnormal neural gamma oscillations[6,27,28]. Whether rhythmic auditory or visual stimulation can restore DOC patients' pathological neural oscillations and consciousness has not been investigated yet. For testing this idea, stimuli carrying highly emotional content may be most suited, as they tend to have relatively strong effects on brain and cognition compared with neutral stimuli[1,30]. For DOC patients, specifically musical stimuli may be effective, as they can evoke salient percepts and strong emotions and have already shown potential for improving DOC patients' arousal and awareness[1]. Therefore, rhythmically modulated auditory music may be an effective sensory stimulus for restoring neural oscillations and consciousness in DOC patients.

Considering the key role of the thalamus in the regulation and integration of multisensory information[31], the most effective approach to target neural oscillations in multiple thalamic nuclei and thalamocortical circuits might be a combination of the aforementioned transcutaneous and auditory modalities. Indeed, a recent study in tinnitus patients shows stronger behavioral and neural effects induced by combined acoustic-electric peripheral nerve stimulation than by acoustic stimulation alone[5], indicating that multimodal stimulation is a promising approach for restoring behavioral and neural deficits.

Based on the findings and considerations above, we reasoned that combining rhythmic TNS and rhythmic musical stimulation ("rhythmic musical-electric TNS" for simplicity) at gamma frequency could provide a highly effective approach to restore thalamocortical oscillations and consciousness in DOC patients. The aims of the present study were two-fold, namely (1) to test whether combined rhythmic musical-

electric TNS can improve consciousness in DOC patients and (2) to explore the neural mechanisms underlying this putative consciousness benefit. We applied rhythmic musical-electric TNS at gamma (40Hz) frequency or non-gamma (28Hz, beta) frequency, or sham stimulation on five consecutive days to 63 DOC patients (42 patients in a minimally conscious state [MCS, a state showing reproducible but inconsistent signs of consciousness] and 21 patients with unresponsive wakefulness syndrome [UWS, a state showing only reflex movements]) (Fig. 1a bottom, 1b). We measured the patients' consciousness before and after the stimulation using behavioral (Coma Recovery Scale-Revised, CRS-R) and neural indicators (electroencephalography, EEG) in a novel auditory-speech paradigm (Fig. 1a top, 1b). We predicted that the stimulation improves patients' consciousness (as reflected by a significant increase in CRS-R score) and neural oscillations (as reflected by a significant increase in rhythmic brain activity), which would render rhythmic musical-electric TNS a strong candidate for DOC treatment. We further predicted that the putative behavioral and neural benefits are coupled (as reflected by a significant correlation between behavioral and neural effects), which would corroborate the notion that neural oscillations and consciousness are functionally coupled.

Results

Positive aftereffect of gamma musical-electric TNS on patients' consciousness

To assess whether musical-electric TNS at gamma frequency (40 Hz) can improve DOC patients' consciousness, we compared patients' CRS-R total score—a widely used behavioral measure of consciousness level—after vs before the gamma stimulation. We found a significant increase in patients' CRS-R total score ($Z = 3.463$, $p = 0.0005$, $r = 0.511$, Fig. 2a). Overall, we found ten out of twenty-three

(43.5%) patients to show an improvement of their diagnosis (an upgrade of diagnosis, e.g., from UWS to MCS; see Methods) after being treated with gamma stimulation (Table 1 and Fig. 2b). These results suggest a positive aftereffect of gamma musical-electric TNS on DOC patients' consciousness.

Positive aftereffect of beta musical-electric TNS on patients' consciousness

To evaluate whether the observed stimulation effect generalizes from gamma to other (non-gamma) stimulation frequencies, we also conducted the experiment in another group of patients receiving the rhythmic musical-electric TNS at a control frequency in the beta range (28 Hz; see Methods). These patients were matched with those above in terms of demographic characteristics (i.e., age, etiology, and time since injury) and had similar CRS-R scores at pretest. We found again a significant increase in patients' CRS-R total score after vs before musical-electric TNS ($Z = 2.601$, $p = 0.009$, $r = 0.411$, Fig. 2a), with five out of twenty (25%) patients showing improvement of diagnosis (Table 1 and Fig. 2b). These results indicate that the positive aftereffect on DOC patients' consciousness can be elicited with rhythmic musical-electric TNS at various frequencies in gamma and beta range.

No significant aftereffect of sham stimulation on patients' consciousness

To rule out that the observed consciousness improvements were caused by potential spontaneous recovery and/or a placebo effect, we further conducted the experiment in a control group receiving only sham stimulation (see Methods). As for the sham-stimulation group, these patients were matched with the gamma-stimulation group in terms of relevant clinical characteristics at pretest. In contrast to the stimulation groups above, the sham-stimulation group showed no significant change in

consciousness: 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, Table 1). This result suggests that the consciousness improvements observed above were caused by the actual musical-electric TNS (and associated neural changes), rather than spontaneous recovery and/or a *placebo* effect.

Estimated size of the stimulation effect on patients' consciousness

To obtain an unbiased measure of the musical-electric TNS effect, we corrected the stimulated patients' pretest-posttest changes for the changes observed after sham stimulation (average pretest-posttest change observed in sham-stimulation group; 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-electric TNS at beta and especially gamma frequency.

Rhythmic musical-electric TNS improves patients' visual and motor abilities

Exploratory analysis of individual CRS-R subscales revealed that the consciousness improvements induced by the musical-electric TNS stimulation 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 $p > 0.1$, false-discovery rate [FDR] corrected, Fig. 2d). These results show that rhythmic musical-electric TNS benefits primarily patients' visual and motor abilities.

Positive frequency-specific aftereffect of rhythmic stimulation on brain activity

To identify the brain processes that putatively mediated the observed effect of rhythmic musical-electric TNS on DOC patients' consciousness, we next explored the effect of the stimulation on patients' rhythmic cortical activity evoked by rhythmic auditory speech (amplitude-modulated at beta or gamma frequency; see Methods).

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 of similar strength in the three groups (no effect of *stimulation type*: gamma ASSR: $F_{2,59} = 0.324$, $p = 0.725$, $\eta^2_p = 0.011$; beta ASSR: $F_{2,59} = 0.466$, $p = 0.630$, $\eta^2_p = 0.016$; 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 ($t_{21} = 3.056$, $p = 0.006$, $d = 0.652$; 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]: $F_{1,21} = 15.557$, $p = 0.0007$, $\eta^2_p = 0.426$). 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* ($F_{1,18} = 3.810$, $p = 0.067$, $\eta^2_p = 0.175$). The latter beta stimulation-induced trend mirrored the above gamma stimulation-induced interaction, suggesting that the rhythmic musical-electric 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*: $F_{1,18} = 0.091$, $p = 0.767$, $\eta^2_p = 0.005$, Fig. 3c, right).

To obtain an unbiased measure of the musical-electric 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; $t_{21} = 4.790$, $p = 9.0 \times 10^{-4}$, $d = 1.021$). 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-electric TNS was significantly stronger on rhythmic responses at the stimulated vs. non-stimulated frequency ($t_{40} = 3.847$, $p = 0.0004$, $d = 0.601$; data not shown). In sum, these neural

results show that the rhythmic musical-electric TNS facilitated DOC patients' rhythmic brain activity especially at the stimulation frequency.

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 from pretest to posttest across all patients. We found a weak significant positive correlation (Spearman's $\rho = 0.252$, $p = 0.026$; Fig. 4), showing that stronger rhythmic brain-activity improvements were accompanied by stronger consciousness improvements. This result suggests that improvements in consciousness are functionally coupled to increases in rhythmic brain activity.

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 linguistic 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 the Supplementary Fig.1-2. In brief, we observed no systematic effect of rhythmic musical-electric 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.

Susceptibility of MCS and UWS patients' consciousness to rhythmic stimulation

To identify DOC patients who benefitted particularly strongly from the rhythmic musical-electric TNS, we explored the sizes of the observed behavioral and neural stimulation effects in DOC patients with different diagnoses at pretest. Fig. 5 shows the same data as Figs. 2c and 3d, now stratified according to the patients' diagnoses (i.e., MCS and UWS) at the time of the pretest. Fig. 5a-b and 5d-e show the behavioral benefit for each clinical entity. We found improved diagnosis after gamma stimulation in eight out of seventeen (47.1%) MCS patients and two out of six (33.3%) UWS patients (Table 1, Fig. 5a, 5d, left). A similar pattern was observed after beta stimulation, showing improved diagnosis in three out of eleven (27.3%) MCS patients and two out of nine (22.2%) UWS patients. These observations suggest that both patient groups may benefit from rhythmic musical-electric TNS, with a stronger benefit for MCS patients. Fig. 5c and 5f show the neural benefit for each diagnostic group. Neural benefits also showed a similar pattern within each group, suggesting that rhythmic musical-electric TNS had similar effects on rhythmic brain activity in MCS and UWS patients. It should be noted that the group of MCS patients was approximately two times larger than the group of UWS patients in our sample; thus our interpretation of these exploratory group-comparison results needs to be treated with caution.

Discussion

The goal of our study was to explore whether (and how) a novel approach—non-invasive rhythmic musical-electric TNS—may improve DOC patients' consciousness. We found that 40 minutes of this stimulation in gamma or beta range (applied repeatedly on five consecutive days) leads to a significant improvement in DOC patients' consciousness. We further found that this stimulation leads to a significant increase in patients' rhythmic brain activity especially at the stimulation frequency. We also observed that the aforementioned 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-electric TNS can improve DOC patients' consciousness and propose frequency-specific enhancement of rhythmic brain activity as a potential neural mechanism.

We found that the musical-electric 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), for which class II evidence has been provided[32]. The most effective tDCS trials so far have achieved consciousness improvement (defined as an increase of minimally one CRS-R subscore) in 43.3% of MCS patients and 8% of UWS patients after a single administration of tDCS above the left dorsolateral prefrontal cortex[2] and in 56.3% of MCS patients after five consecutive administrations of this stimulation[33]. Applying the same consciousness-improvement criterion to our data, we find *post hoc* a significant improvement in 58.8% of our MCS patients and 33.3% of UWS patients after gamma stimulation, and in 41.1% of MCS patients and 22.2% of UWS patients

after beta stimulation. Thus, in direct comparison with previous non-invasive electric brain-stimulation methods, our rhythmic musical-electric TNS especially in the gamma range constitutes a strong candidate for effective DOC treatment. It should be noted that patients in the present and aforementioned studies differed in their post-injury time. In the studies by Thibaut and colleagues, post-injury time varied from 18 days to 30 years, whereas in our study it varied from 1 to 11 months. As DOC patients often recover within the first three (nontraumatic) to twelve (traumatic) months after injury, patients with a shorter post-injury time may have better prognosis[34]. 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.

We further observed that the rhythmic musical-electric 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[33,35], transcranial magnetic stimulation[36] and vagus-nerve stimulation[3]. The strong effect on visual abilities may be explained by the fact that visual signs constitute a highly sensitive behavioral indicator of consciousness[37]. The strong effect on patients' motor abilities may be due to the non-invasive electric stimulation effects spreading to the motor cortex[19], 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-electric TNS may be more effective for MCS patients than UWS patients, which is in line with other treatment studies reporting larger effects in MCS patients[2,38]. 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[39,40]. This putatively stronger connectivity in MCS patients may have allowed the neural effects of electric stimulation to spread more widely across the MCS patient's brain, which may have resulted in more widespread plasticity and stronger consciousness recovery[41].

A major finding from our study is that musical-electric 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[42] and the duration of such aftereffects seems to be positively related to the duration of the stimulation (tACS[43]; tDCS[44]). Moreover, the strength of these aftereffects may be positively related to the strength of the instantaneous effects of the stimulation[45,46]. 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[4,5,28].

A candidate mechanism underlying the aftereffects of electric brain stimulation is spike-timing dependent plasticity[43]. External rhythmic stimulation may "reshape" neuronal circuits by inducing adaptions in temporal patterns of synaptic activity[47,48]. These adaptations may persist and reverberate until after the stimulation, resulting in a long-lived rhythmic aftereffect[49,50]. 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[48]. 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-electric 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[51]. Another study observed a stronger correlation between consciousness and rhythmic brain activity at 40 Hz than at other frequencies[16]. 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[51].

Important questions to be addressed in future research concern the relative effectiveness of the different (acoustic vs electric) stimulation types and the relation of their duration and aftereffects. For this, the acoustic and electric stimulation should be applied in isolation and its duration and intensity should be systematically varied

to identify the minimum dose required to achieve a treatment effect of a given strength. Moreover, it would be informative to conduct multiple consecutive neural assessments during the posttest phase to assess the persistence of the aftereffects over a longer time course.

Considering rhythmic musical-electric 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 related to the frequency-specific increase in rhythmic brain activity. 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[51]. As mentioned in the Introduction, direct electric stimulation of the thalamus may improve consciousness[12-14] and our rhythmic musical-electric 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.

A potential mechanism by which rhythmic brain activity may improve consciousness is through the modulation of the gamma-aminobutyric acid (GABA) neurotransmitter system. Reduced consciousness in DOC patients has been linked to reduced gamma brain activity[16]. Abnormal gamma-activity levels may reflect imbalance between neural excitation and neural inhibition[52], which are controlled by inhibitory GABA neurotransmitters[53]. Thus, it is conceivable that our gamma stimulation rebalanced neural excitation and inhibition through modulation of the GABA system[54], thereby leading to the observed increases in gamma activity and consciousness (similar to the action of an "awakening" drug such as Zolpidem[55]). Testing this idea would require to assess the effect of gamma TNS on GABA neurotransmitter levels using e.g., magnetic resonance spectroscopy.

Intriguingly, 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[56]) 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[57,58]. Moreover, detection of changes in repeated utterances has been shown to lead to increased gamma activity[59]. 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[60]. Contrarily, neural tracking of sentential structure is not observable during sleep[61].This suggests that detection of semantic deviations reflects an automatic process[60,62], whereas tracking of sentential structure relies on continuous comprehension using top-down lexical knowledge[63]. 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.

In conclusion, rhythmic musical-electric TNS can improve consciousness in DOC patients, especially MCS patients. The benefit seems to be facilitated by a frequency-specific enhancement of rhythmic neural processing. Stimulation at gamma frequency may be the most promising approach, as it resulted in the largest improvements in CRS-R scores in our study and seems to additionally improve patients' linguistic abilities. Future studies should disentangle the relative

contributions of the acoustic and electric stimulation and characterize the relation between their duration and aftereffects.

Methods

Patients

Seventy-three patients diagnosed with DOC were recruited from **** Hospital. All patients met the following criteria: (1) diagnosis of UWS or MCS based on CRS-R, (2) time post-injury ranging from one to twelve months, (3) no history of acquired brain injury or psychiatric or neurological diseases, (4) no major skull-bone or brain-tissue defects (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 and registered in a publicly accessible clinical trial database on ****. Informed consent was obtained from the patients' legal surrogates. Patients were assigned to three groups matched for demographic characteristics (i.e., age, etiology and time since injury) and CRS-R score at pretest (see section *Assessment*). Sixty-three of the 73 recruited patients completed the study (seven patients were released from the hospital and three others showed excessive body movements during the pretest phase). Patients' demographic and clinical characteristics are shown in Table 1.

Assessment

Each patient underwent three phases: pretest, stimulation, and posttest. The pretest phase and posttest phase involved the administration of the same set of behavioral and neural assessments, as illustrated in Fig. 1a top.

Behavioral assessment: CRS-R

Patients' consciousness was assessed using the CRS-R, a validated and commonly used measure of consciousness in DOC patients[64]. The CRS-R consists of six subscales assessing respectively the patient's auditory function, visual function, motor function, oromotor function, communication ability, and arousal. 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[65]. The clinicians and the patients' legal surrogates were blinded to the treatment condition (see section *Study design and Experimental procedure*). 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).

Neural assessment: EEG

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.

Auditory paradigm for neural assessment

An auditory paradigm with speech stimuli was used to facilitate assessment of neural rhythmic activity and linguistic processes. The speech stimuli were repetitive quadruples 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 quadruples (*"one-two-three-four"* or *"east-south-west-north"*, which is the conventional order in Chinese). We refer to these quadruples 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). 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 (50 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. Uneven-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.

Stimulation

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

Acoustic stimulation: music

Acoustic stimulation consisted of excerpts from ten pieces of popular 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 (43 dB SPL) simultaneously with the electric or sham stimulation (see next section).

Electric stimulation: TNS

Electric 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[26], 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 electric 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 acoustic and electric stimulation were ramped down after 0.5 min to remain turned off for the remaining 39.5 min. Note that patients may have been able to notice the muting of the acoustic stimulation in this condition.

Study design and experimental procedure

The study used a mixed 2×3 design with the within-subject factor *time* (pretest, posttest) and the between-subject factor *stimulation* (gamma, beta, sham). Fig. 1b illustrates the schedule of the pretest phase, stimulation phase, and posttest phase. 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. For the gamma- and beta-stimulation groups, gamma and beta musical-electric TNS was administered; for the sham-stimulation group, sham stimulation was administered. 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.

Data analysis

EEG data preprocessing

Data preprocessing and analysis was performed offline using EEGLAB 2019.1[66] 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[67]. For this analysis the data were band-pass filtered between 1 Hz and 40 Hz using a linear-phase finite impulse response (FIR) 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[68]. The weights of the non-artifactual components were reapplied to the original unfiltered channel data[69]. 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).

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 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.

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.

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[70]. 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.

Extraction of an unbiased measure of the musical-electric TNS effect

To extract the effect of the stimulation phase on patients' consciousness and brain activity, behavioral and neural data obtained during the posttest were subtracted from those obtained during the pretest. This was done for each stimulation group (gamma-stimulation group, beta-stimulation group, and sham-stimulation group). To obtain an unbiased measure of the effect of the actual rhythmic musical-electric TNS, 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.

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 Leven'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).

Data availability

All data and code are available upon reasonable request.

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Table 1. Demographic and clinical characteristics of the DOC patients.

Stimulation	Patient number	Age (years)	Gender	Etiology	Time since injury (months)	CRS-R subscores at pretest	CRS-R total score at pretest	Diagnosis at pretest	CRS-R subscores at posttest	CRS-R total score at posttest	Diagnosis at posttest	Improvement of diagnosis
Gamma	1	70	M	CVA	4.0	011102	5	UWS	231102	9	MCS-	**Y**
	2	38	F	CVA	3.0	132102	9	MCS-	343012	13	MCS+	**Y**
	3	68	M	TBI	8.5	112102	7	UWS	112102	7	UWS	N
	4	48	M	CVA	2.0	031102	7	MCS-	032102	8	MCS-	N
	5	44	F	CVA	2.0	112102	7	UWS	122102	8	MCS-	**Y**
	6	33	M	CVA	3.5	132102	9	MCS-	334102	13	MCS+	**Y**
	7	48	M	CVA	2.0	135102	12	MCS-	456112	19	EMCS	**Y**
	8	56	M	TBI	1.0	234102	12	MCS-	456112	19	EMCS	**Y**
	9	62	F	ABI	5.5	111100	4	UWS	112100	5	UWS	N
	10	42	M	ABI	5.5	232102	10	MCS-	453123	18	EMCS	**Y**
	11	32	M	CVA	1.5	122002	7	MCS-	453123	18	EMCS	**Y**
	12	69	M	CVA	1.5	132102	9	MCS-	232102	10	MCS-	N
	13	37	M	CVA	7.0	032002	7	MCS-	032102	8	MCS-	N
	14	41	M	CVA	3.5	131102	8	MCS-	232102	10	MCS-	N
	15	63	F	CVA	1.5	222102	9	MCS-	132102	9	MCS-	N
	16	24	M	CVA	4.0	132102	9	MCS-	230102	8	MCS-	N
	17	47	F	CVA	10.0	111102	6	UWS	111102	6	UWS	N
	18	44	F	TBI	8.0	232102	10	MCS-	445112	17	MCS+	**Y**
	19	52	M	CVA	5.0	112100	5	UWS	112100	5	UWS	N
	20	65	F	CVA	4.0	222102	9	MCS-	222102	9	MCS-	N
	21	43	F	TBI	3.5	232102	10	MCS-	332212	13	MCS+	**Y**
	22	70	F	TBI	7.0	222102	9	MCS-	222102	9	MCS-	N
	23	72	F	TBI	8.5	232102	10	MCS-	233102	11	MCS-	N
Beta	24	40	F	ABI	9.0	352102	13	MCS+	354102	15	MCS+	N
	25	66	F	ABI	2.0	122102	8	MCS-	112102	7	UWS	N
	26	65	M	TBI	11.0	233102	11	MCS-	356112	18	EMCS	**Y**
	27	47	M	ABI	10.5	232102	10	MCS-	122102	8	MCS-	N
	28	70	M	CVA	5.0	114102	9	MCS-	124102	10	MCS-	N
	29	41	M	TBI	6.5	112002	6	UWS	322112	11	MCS+	**Y**
	30	79	M	TBI	3.5	211102	7	UWS	212102	8	UWS	N
	31	47	M	TBI	1.5	235102	13	MCS-	345112	16	MCS+	**Y**
	32	67	M	ABI	1.5	215102	11	MCS-	225102	12	MCS-	N
	33	79	M	TBI	6.0	115102	10	MCS-	334102	13	MCS+	**Y**
	34	56	F	TBI	6.5	212102	8	UWS	212102	8	UWS	N
	35	39	M	TBI	1.5	112102	7	UWS	212102	8	UWS	N
	36	31	M	CVA	1.0	212102	8	UWS	212102	8	UWS	N
	37	59	M	CVA	1.0	232102	10	MCS-	235102	13	MCS-	N
	38	44	F	CVA	2.0	334102	13	MCS+	354112	16	MCS+	N
	39	79	F	ABI	2.0	112102	7	UWS	212102	8	UWS	N
	40	32	M	CVA	2.0	102100	4	UWS	102100	4	UWS	N
	41	61	M	CVA	1.0	212100	6	UWS	112100	5	UWS	N
	42	50	M	ABI	1.0	212102	8	UWS	222102	9	MCS-	**Y**
	43	37	M	CVA	1.0	232102	10	MCS-	232102	10	MCS-	N
Sham	44	46	F	ABI	3.0	101102	5	UWS	101102	5	UWS	N
	45	54	M	CVA	4.0	132102	9	MCS-	232102	10	MCS-	N
	46	49	M	CVA	4.0	132102	9	MCS	112102	7	UWS	N
	47	74	M	TBI	7.0	212102	8	UWS	212102	8	UWS	N
	48	39	M	TBI	3.0	212102	8	UWS	212102	8	UWS	N
	49	56	F	TBI	5.0	222102	9	MCS-	232102	10	MCS-	N
	50	70	M	TBI	8.5	355102	16	MCS+	232102	10	MCS-	N
	51	66	M	TBI	1.0	212102	8	UWS	212102	8	UWS	N
	52	60	F	CVA	1.0	232102	10	MCS-	232102	10	MCS-	N
	53	57	F	ABI	3.0	212102	8	UWS	212102	8	UWS	N
	54	47	F	CVA	11.0	101102	5	UWS	111102	6	UWS	N
	55	24	M	CVA	5.0	231102	9	MCS-	231102	9	MCS-	N
	56	65	F	CVA	5.0	222102	9	MCS-	232102	10	MCS-	N
	57	51	M	CVA	1.0	232102	10	MCS-	233102	11	MCS-	N
	58	56	M	ABI	1.0	232102	10	MCS-	232102	10	MCS-	N
	59	44	F	CVA	4.0	222102	9	MCS-	222102	9	MCS-	N
	60	70	M	CVA	6.0	335112	15	MCS+	335112	15	MCS+	N
	61	37	M	CVA	9.0	232102	10	MCS-	232102	10	MCS-	N
	62	40	F	ABI	10.0	234102	12	MCS-	234102	12	MCS-	N
	63	68	M	TBI	11.0	132102	9	MCS-	222102	9	MCS-	N

The six digits in the seventh column represent CRS-R subscale scores (auditory function, visual function, motor function, oromotor function, communication ability, and arousal, respectively) obtained during the pretest measurement yielding the

"best" diagnosis (i.e., highest consciousness). Subscale scores that determined an MCS (including MCS+ and MCS-) or EMCS diagnosis are underlined. Analogously, the tenth column shows CRS-R subscale scores obtained from the best posttest measurement. The final column highlights patients whose best diagnosis improved from pretest to posttest phase.

CVA, cerebrovascular accident; TBI, traumatic brain injury; ABI, anoxic brain injury; M, male; F, female; MCS, minimally conscious state; UWS, unresponsive wakefulness syndrome; EMCS, emergence from MCS; CRS-R, Coma recovery scale-revised.

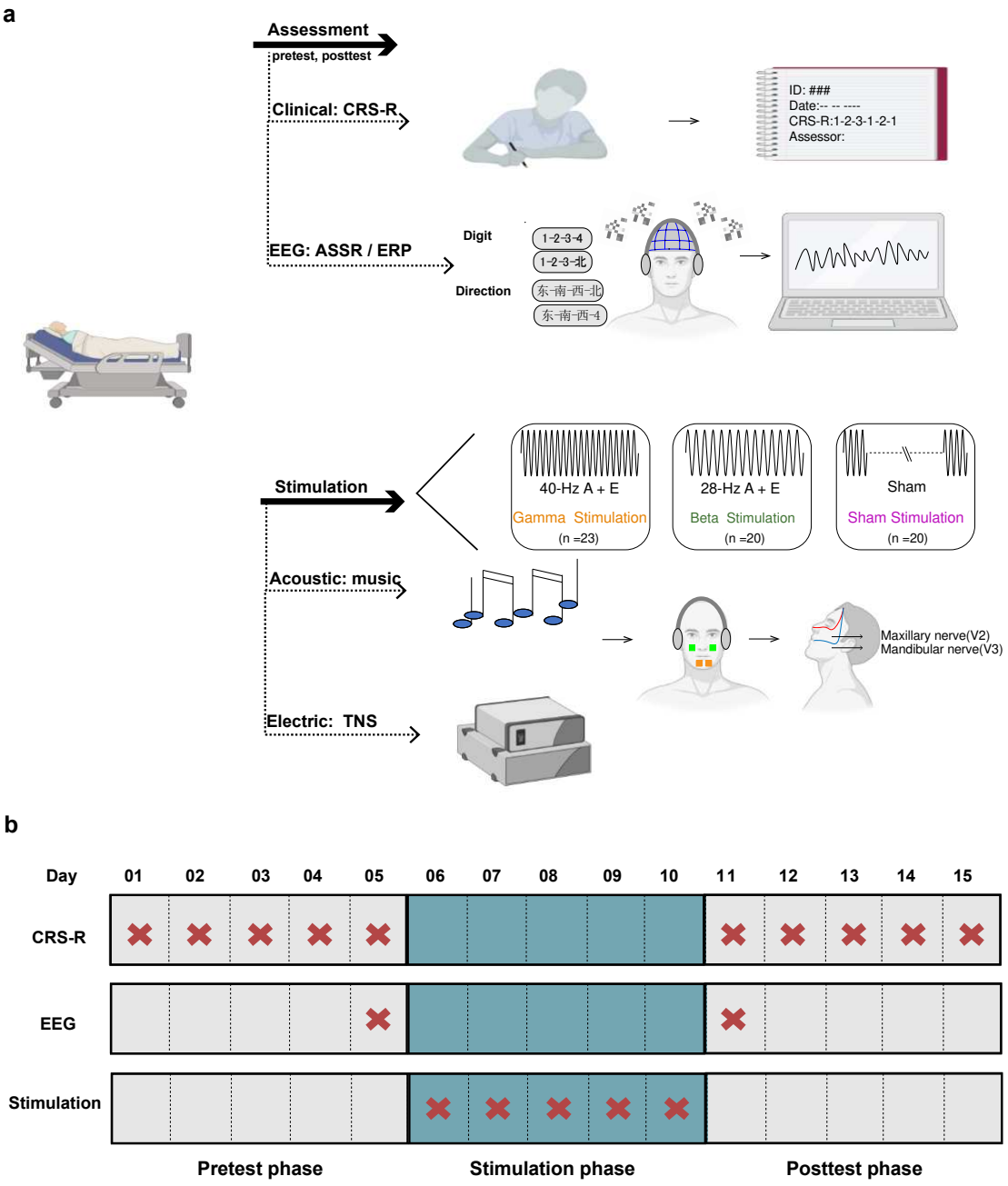

Fig. 1. Study design and experimental procedure.

(a). Schematic of assessments and musical-electric TNS. DOC patients' consciousness was assessed by two experienced blinded clinicians using the CRS-R. Patients' neuroelectric brain activity was assessed by measuring 64-channel EEG during the presentation of continuous rhythmic auditory Chinese speech. The rhythmic musical-electric TNS involved the simultaneous application of auditory

music via earphones and electric current via electrodes attached to the middle and lower parts of the patient's face (see green and orange squares). Both acoustic and electric 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 (gray), stimulation (cyan), and posttest (gray). The treatment phase involved daily administration of 40 min of rhythmic musical-electric 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.

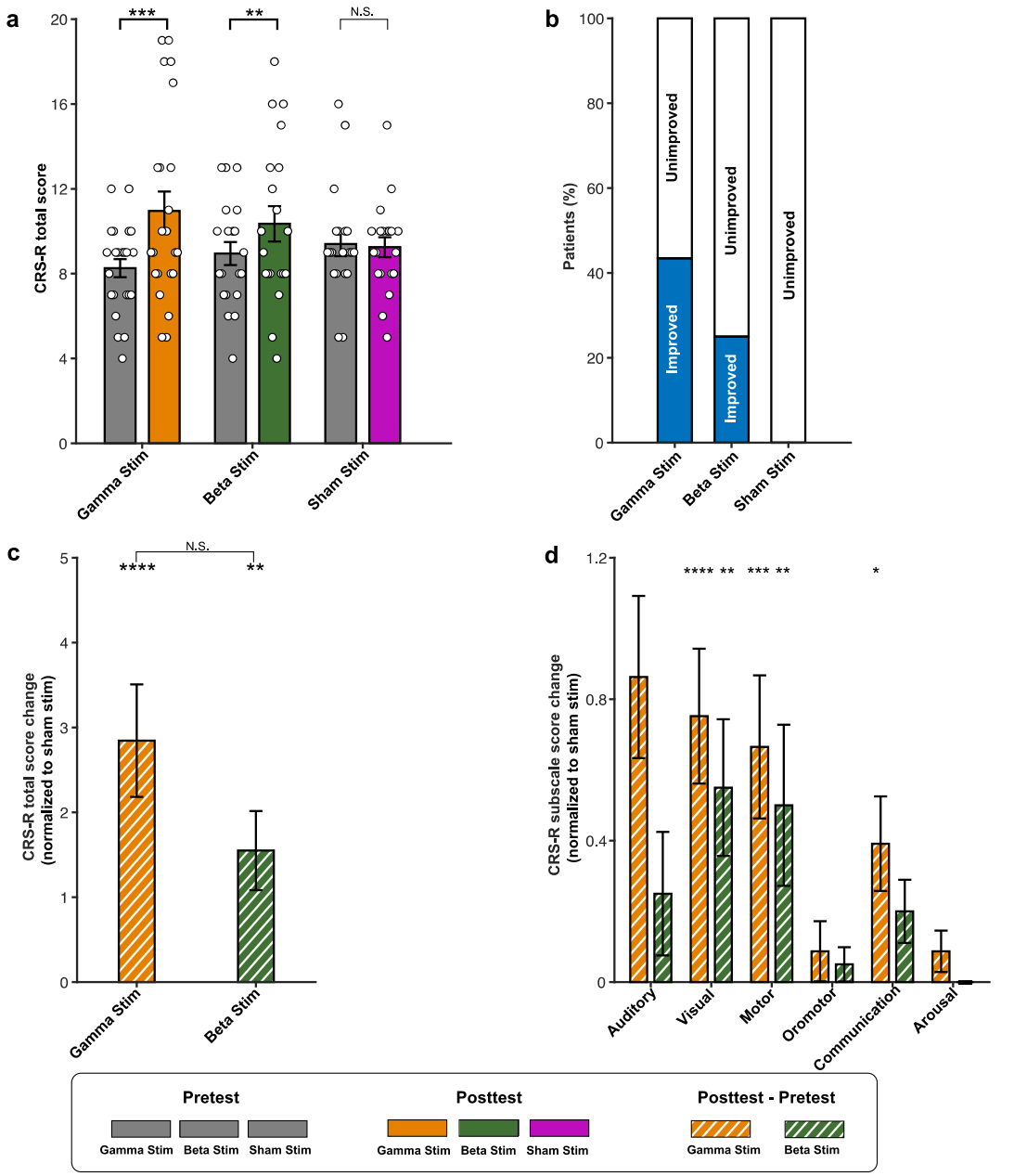

Fig. 2. Consciousness level in DOC patients before and after rhythmic musical-electric TNS or sham stimulation.

(a). CRS-R total scores of DOC patients in pretest (gray) and posttest (hue). The leftmost pair of bars represents the outcome 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-electric 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 abilities. Gamma stimulation also induced a large change in auditory ability; however, this change was observed only in a small subset of patients, thus missing significance at the group level (corrected $p = 0.054$, non-parametric test).

Data are presented as mean ± sem across participants (a, c, d), except for white dots in panel a, which represent individual scores of patients. n.s. non-significant, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

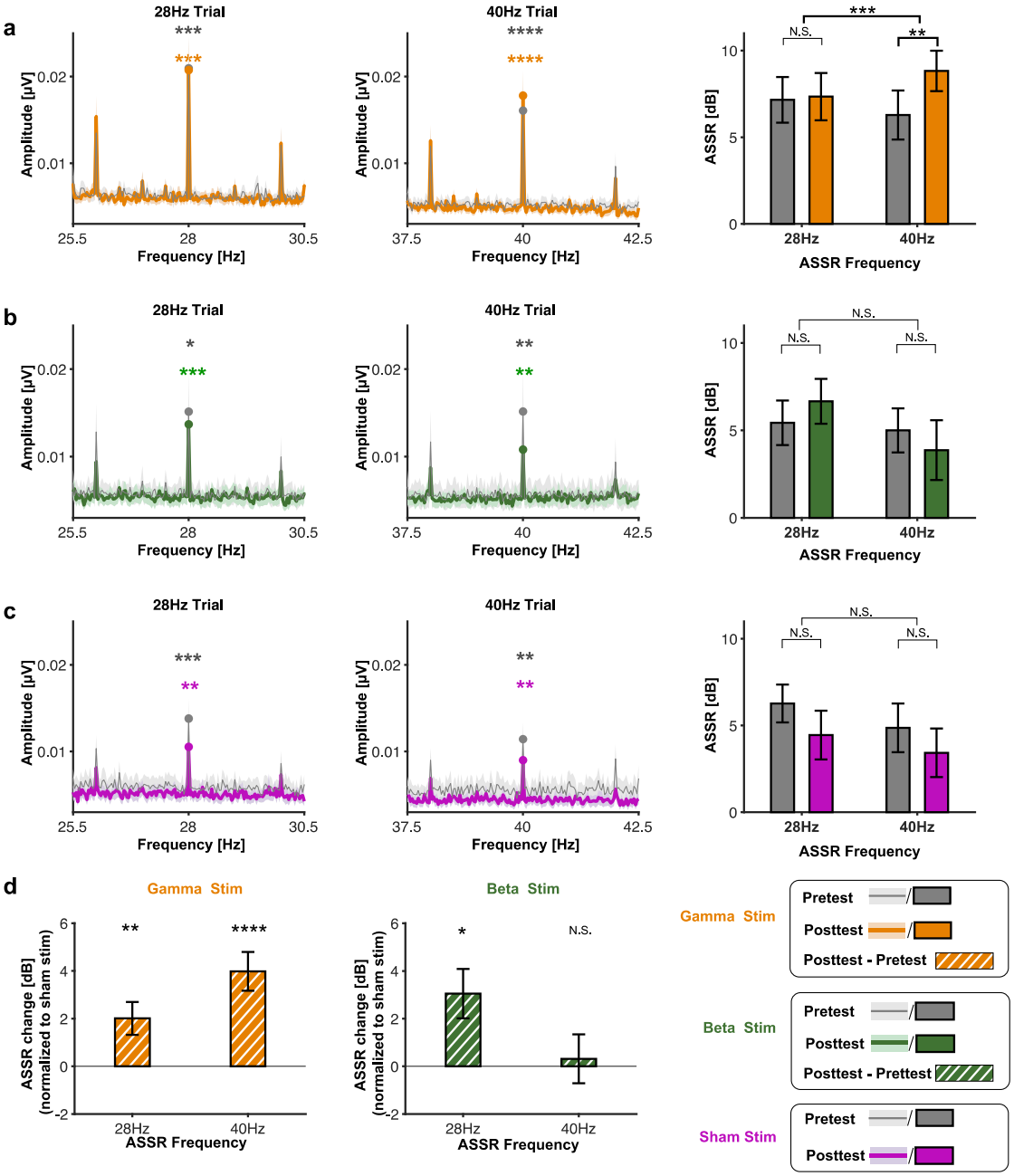

Fig. 3. Rhythmic brain responses to amplitude-modulated speech in DOC patients before and after rhythmic musical-electric TNS or sham stimulation. (a). Spectral response to beta (28 Hz, left) or gamma (40 Hz, center) amplitude-modulated speech in pretest (gray) and posttest (orange) for the gamma-stimulation group. Asterisks indicate significant responses at the test frequency relative to the noise floor (average responses in the surrounding frequency bins). 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). Gamma stimulation and beta stimulation had significantly positive effects on rhythmic brain activity especially at the stimulation frequency. Data are presented as mean ± sem across participants. n.s. non-significant, *p < 0.05, **p <0.01, ***p < 0.001, ****p<0.0001.

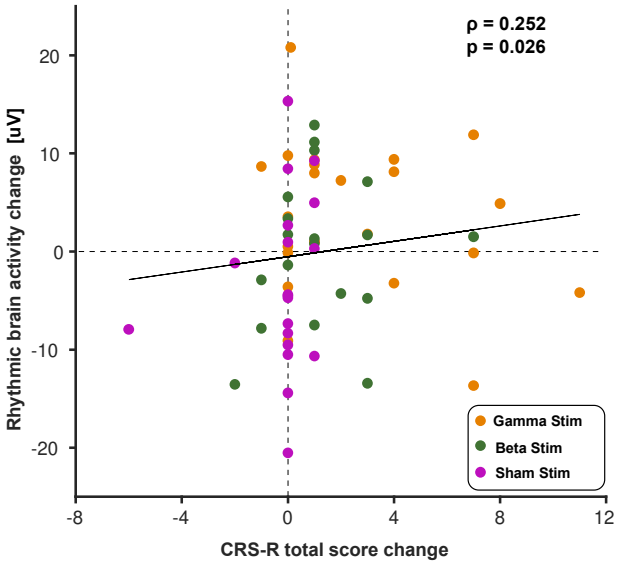

Fig. 4. Relation between changes in consciousness and changes in rhythmic brain activity in DOC patients.

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.

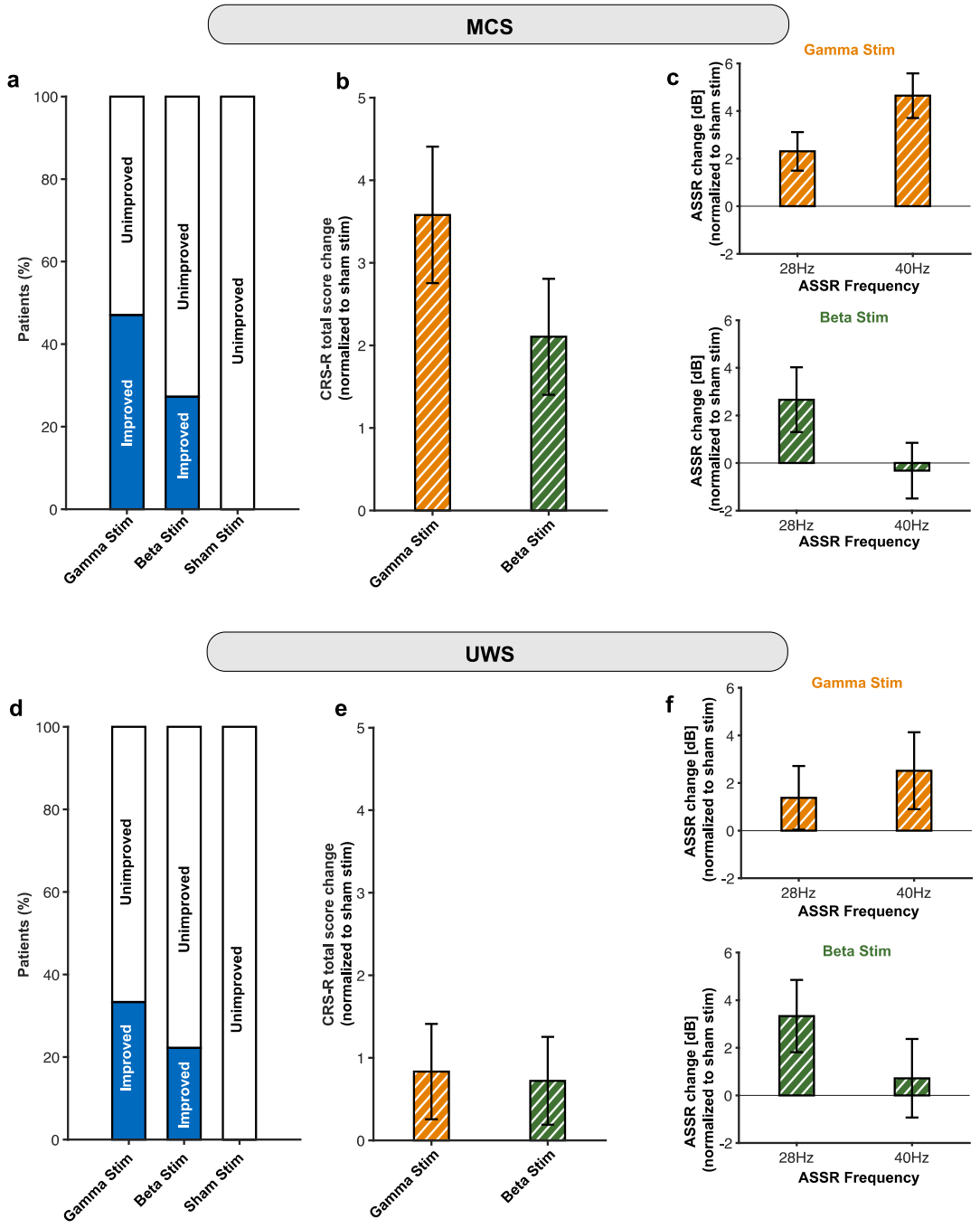

Fig. 5. Changes in consciousness level and rhythmic brain activity induced by rhythmic musical-electric TNS in MCS and UWS patients.

(a-c). Same as Figs. 2b, 2c, and 3d (respectively), but for patients diagnosed with MCS at pretest (gamma-stimulation group: N = 17 in panels a, b; N = 16 in panel c; beta-stimulation group: N = 11; sham-stimulation group: N = 13 in panels a, b; N = 12 in panel c).

(d-f). Same as panel a, but for patients diagnosed with UWS at pretest (gamma-stimulation group: N = 6; beta-stimulation group: N = 9 in panels d, e; N = 8 in panel f; sham-stimulation group: N = 7).

MCS and UWS patients showed an overall similar pattern of behavioral and neural changes, with larger behavioral changes in MCS patients. However, these descriptive differences may be partially attributed to a difference in sample size.

Data in panels b, c, e, and f are presented as mean ± sem across participants.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryResults.pdf