

Modulating consciousness with acoustic-electric stimulation

Citation for published version (APA):

Wu, M. (2023). *Modulating consciousness with acoustic-electric stimulation*. [Doctoral Thesis, Maastricht University]. Maastricht University. <https://doi.org/10.26481/dis.20230914mw>

Document status and date:

Published: 01/01/2023

DOI:

[10.26481/dis.20230914mw](https://doi.org/10.26481/dis.20230914mw)

Document Version:

Publisher's PDF, also known as Version of record

Please check the document version of this publication:

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Modulating consciousness with acoustic-electric stimulation

Min Wu

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Layout: Min Wu

Cover design: Min Wu & Sheng Kuang

Production: ProefschriftMaken, www.proefschriftmaken.nl

ISBN: 978-94-6469-524-3

Modulating consciousness with acoustic-electric stimulation

DISSERTATION

to obtain the degree of Doctor at the Maastricht University,
on the authority of the Rector Magnificus, Prof.dr.Pamela Habibović
in accordance with the decision of the Board of Deans,
to be defended in public
on Thursday, 14th of September 2023 at 10:00 hours
by

Min Wu

Supervisor(s)

Dr. Lars Riecke

Prof. Dr. Elia Formisano

Dr. Bettina Sorger

Assessment Committee

Prof. Dr. Alexander Sack (Chair)

Prof. Dr. David Linden

Dr. Aurore Thibaut (Coma Science Group, University of Liège, Belgium)

Dr. Benedikt Zoefel (Brain and Cognition Research Centre, CNRS, France)

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Chapter 1

General introduction

1 Consciousness and disorders of consciousness

Consciousness is around everywhere in our daily lives, manifesting through experiences such as seeing an object, hearing a sound, or feeling pain. It comprises two fundamental components: wakefulness and awareness. Wakefulness refers to the global arousal states, such as coma and sedation, while awareness refers to the content of consciousness, such as the perception of object shapes (Bayne, Hohwy, & Owen, 2016; Seth & Bayne, 2022). Consciousness states fluctuate during sleep, sedation, or under pathological conditions. For instance, consciousness disappears during dreamless sleep and reemerges during lucid dreaming. In patients with severe brain injuries, consciousness may become impaired, resulting in *disorders of consciousness* (DOC). These disorders comprise a range of conditions, including coma, *unresponsive wakefulness syndrome* (UWS) and *minimally conscious state* (MCS).

Coma is a transient state characterized by the absence of both wakefulness and awareness (Laureys, Owen, & Schiff, 2004). Approximately 10-15% of patients who awaken from coma but remain unresponsive progress into the state of UWS (Jennett & Plum, 1972; Levin et al., 1991), previously also known as *vegetative state* (VS) (Laureys et al., 2010). These patients can perform reflexive movements but show no voluntary responses or signs of self-awareness or environmental awareness. Patients who exhibit inconsistent yet detectable signs of awareness are considered to be in MCS (Giacino et al., 2002). Given that coma is a transient state, UWS and MCS have gained more attention and research for their monitoring and treatment. Therefore, the term DOC typically refers to chronic conditions, including UWS and MCS.

Understanding the neural mechanisms underlying consciousness is important in the field of neuroscience, as it can pave the way for consciousness assessment and restoration. With the development of advanced neuroimaging techniques, an increasing number of studies has revealed that consciousness maintenance depends on the proper functioning of various components of neural systems (Dehaene & Changeux, 2011; Gosseries, Di, Laureys, & Boly, 2014; Gosseries, Zasler, & Laureys, 2014; Sanchez, Hartmann, Fusca, Demarchi, & Weisz, 2020; Zhao et al., 2019). Two approaches are commonly used to identify *neural correlates of consciousness*, defined as the minimal neural mechanisms that are necessary and sufficient for experiencing consciousness (Crick & Koch, 1990): the first commonly used approach involves contrasting conscious versus unconscious processes in response to identical stimuli; the second complementary approach compares brain responses between individuals exhibiting reduced consciousness, such as patients with DOC, and those with normal consciousness, such as healthy human individuals (Forster, Koivisto, & Revonsuo, 2020; Koch, Massimini, Boly, & Tononi, 2016).

A wide range of candidate neural correlates of consciousness have been proposed over time. In 1949, Moruzzi and Magoun first proposed the ascending reticular activating system (ARAS) in the brainstem and its role in consciousness (Moruzzi & Magoun, 1949). Subsequently, other brain regions, such as the thalamus, prefrontal cortex and parietal cortex, have been identified as key regions for consciousness (Llinas, Ribary, Contreras, & Pedroarena, 1998; Seth & Bayne, 2022). These empirical findings have contributed to the development of various theories of consciousness. For instance, the *global workspace theory* hypothesizes that consciousness is associated with a wide spread of cortical activity, with a particular emphasis on the central role of frontoparietal cortical regions (Baars, 1993; Baars, 2005; Mashour, Roelfsema, Changeux, & Dehaene, 2020). Comparatively, the *recurrent processing theory* claims that the recurrent processing in the sensory cortices alone is sufficient for consciousness (Lamme & Roelfsema, 2000). In addition to structural neural correlates, neurophysiological correlates such as brain oscillations have gained considerable attention. It is widely acknowledged that the neural information from multiple regions is integrated by a cooperative network of interconnected neurons, resulting in synchronized neural activity. Low gamma-band (30-50 Hz) oscillation has been proposed as a biological basis for feature binding and perceptual experience (Engel, Fries, & Singer, 2001; Keil, Müller, Ray, Gruber, & Elbert, 1999). Furthermore, evidence shows that the thalamocortical system plays a central role in consciousness by modulating

40-Hz brain oscillations (Calabro, Cacciola, Bramanti, & Milardi, 2015; Koch et al., 2016; Llinas et al., 1998; Llinas, Urbano, Leznik, Ramirez, & van Marle, 2005). These insights on neural correlates offer promising avenues for assessing consciousness and identifying potential therapeutic targets.

Nevertheless, the accurate assessment and efficient restoration of consciousness remain significant challenges in both scientific research and clinical practice. To overcome these challenges, researchers have developed various diagnostic tools and therapeutic interventions. In Sections 2 and 3 of this introduction, I will review the current state of knowledge and progress made in the fields of diagnosis and treatment of disorders of consciousness, while in Section 4, I will discuss a potential novel approach for consciousness improvement.

2 Review of consciousness assessment

2.1 Behavioral indicators

The direct assessment of consciousness levels¹ primarily relies on behavioral measurements using clinical consensus and behavioral scales. The most commonly used scale is *Glasgow Coma Scale* (GCS). This scale was designed to assess the depth of impaired consciousness in the acute stage, involving the assessments of motor responsiveness, verbal performance, and eye opening (Teasdale & Jennett, 1974). However, a number of limitations have been identified applying the GCS. Firstly, complications such as endotracheal intubation, eyelid swelling and sedation can hamper GCS assessment, making it untestable (Gabbe, Cameron, & Finch, 2003). Secondly, the three subscales carry distinct information and simply summing them together may result in the loss of important information and potential misinterpretation (Matis & Birbilis, 2008). Thirdly, the GCS does not incorporate brainstem reflexes, which are considered important indicators of basic consciousness arousal systems (Matis & Birbilis, 2008).

To address these limitations, Wiejicks and colleagues developed a new behavioral scale called the *Full Outline of Unresponsiveness* (FOUR) (Wijicks, Bamlet, Maramattom, Manno, & McClelland, 2005). This scale comprises four components, namely eye opening, motor response, brainstem reflexes, and respiration, with each component having a maximal score of four. Several studies have compared the FOUR and GCS, yielding varied results. For example, the FOUR score has been demonstrated to be more reliable than the GCS in assessing patients with traumatic brain injury due to its excellent interrater reliability (Iyer et al., 2009). However, other studies have shown that the FOUR is not superior to GCS in evaluating consciousness levels, making it insufficient to replace the GCS (Eken, Kartal, Bacanlı, & Eray, 2009; M. Fischer et al., 2010).

The Coma-recovery scale-revised (CRS-R) is a reliable tool capable of differentiating different altered states of consciousness (Giacino, Kalmar, & Whyte, 2004). The scale 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 allowing for a quantitative assessment. Based on subscale scores, patients are diagnosed as either UWS, MCS (including two subcategories, termed MCS+ and MCS- based on the presence or absence of language function), or emergence from MCS (EMCS), allowing for a qualitative assessment. Using CRS-R assessment, up to 43% of UWS diagnoses based on clinical consensus were found to be in MCS (Schnakers et al., 2009). Currently, CRS-R remains the gold standard for consciousness assessment and diagnosis determination (Giacino et al., 2004). Repeated assessments over time are

¹ I agree with the perspective presented by Bayne et al. that assessing levels of consciousness in terms of global states can be challenging. Therefore, it is essential to evaluate consciousness based on multiple dimensions (Bayne et al., 2016).

strongly recommended, as consciousness levels often fluctuate in patients with DOC (Wannez et al., 2017). Nonetheless, due to the motor impairments in DOC patients, the lack of overt responsiveness may lead to the underestimation of consciousness. This encourages the development of electroencephalographic and neuroimaging techniques that rely on objective measures (Edlow et al., 2017; Kondziella et al., 2020).

2.2 The neurophysiological approach using electroencephalography

2.2.1 Resting state

Electroencephalography (EEG) is a practical, cost-effective neurophysiological technique that can be administered at the bedside and is commonly used for consciousness assessments. According to the “ABCD” model used to assess thalamocortical integrity, the EEG power spectrum is classified into four categories: A-type, characterized by predominantly delta activity (<1 Hz); B-type, characterized by theta activity (5-9 Hz); C-type, predominantly exhibiting theta (5-9 Hz) and beta (20-35 Hz) activity; D-type, predominantly exhibiting alpha (8-13 Hz) and beta (20-35 Hz) activity. A transition from UWS to MCS is associated with an EEG spectral shift from B-type to C- or D-type (Edlow, Claassen, Schiff, & Greer, 2021). Converging evidence suggests that patients with low consciousness levels exhibit enhanced activity in lower frequencies (delta and theta) and suppressed activity in higher frequencies (alpha, beta, gamma) (Chennu et al., 2014; Leon-Carrion, Martin-Rodriguez, Damas-Lopez, Barroso y Martin, & Dominguez-Morales, 2008; Naro, Bramanti, Leo, Cacciola, et al., 2016).

In addition to spectral power, sufficient complexity is essential for consciousness. Many studies have reported that the global complexity of EEG signals is higher in MCS patients than in those with UWS (Gosseries et al., 2011; Lee, Hussein, & McKeown, 2019; Liu et al., 2023). Recent research has segmented ongoing EEG into short momentary segments, known as EEG microstates (Bréchet & Michel, 2022; Lei et al., 2022; Zappasodi et al., 2019). These microstates are considered the basic building blocks of consciousness (Bréchet & Michel, 2022; Michel & Koenig, 2018). Studies have shown that a longer duration of the microstate is associated with a lower level of consciousness (Gui et al., 2020), and that each microstate corresponds to a specific brain network (Zhang et al., 2023).

Moreover, numerous resting-state functional-connectivity measurements, such as coherence, phase-lag index (PLI), symbolic mutual information (SMI), and phase-locking value (PLV) have been used to quantify the synchronization and communication between distinct neural populations in the assessment of consciousness (Bourdillon et al., 2020; Chennu et al., 2017; Chennu et al., 2014; Rizkallah et al., 2019). In comparison to UWS, MCS patients generally exhibit decreased functional connectivity in the delta band and increased functional connectivity in other higher frequency bands (i.e., theta, alpha, beta), particularly in the frontocentral and frontoposterior regions (Chennu et al., 2017; Lehembre et al., 2012; Naro et al., 2018; Sitt et al., 2014; Stefan et al., 2018). These findings align with those from studies using spectral power analysis, with the exception of changes in the theta band (although some studies also report an excess of theta power in MCS; see review by Duszyk-Bogorodzka, Zieleniewska, & Jankowiak-Siuda, 2022).

It is worth noting that functional connectivity in the gamma band has been associated with visual, tactile and olfactory consciousness (Gallotto, Sack, Schuhmann, & de Graaf, 2017; Gray, König, Engel, & Singer, 1989; Meador, Ray, Echaz, Loring, & Vachtsevanos, 2002; Mori, Manabe, Narikiyo, & Onisawa, 2013). However, its role in patients with DOC remains relatively unexplored. This knowledge gap is a major focus that is addressed in Chapter 5, providing a more comprehensive understanding of the gamma functional connectivity in patients with DOC.

2.2.2 Passive EEG paradigms

2.2.2.1 Oddball paradigm

The oddball paradigm is simple yet frequently used for consciousness assessment in DOC patients, in which a deviant auditory stimulus is embedded in a sequence of standard auditory stimuli (Fischer, Luaute, & Morlet, 2010; Kotchoubey et al., 2005; Perrin et al., 2006; Qin et al., 2008). These stimuli evoke multiple brain responses called event-related potentials (ERPs). For instance, the N100 component is the first negative-going component that peaks around 100 ms after the onset of a stimulus, indexing primary sensory perception even without task demands. Although the N100 is found more frequently in MCS than in UWS patients, it lacks sensitivity to reliably discriminate between MCS and UWS on an individual level (Kotchoubey et al., 2005; Wu et al., 2020).

Mismatch negativity (MMN) is another negative-going waveform calculated by subtracting responses to the standard stimuli from those to deviant stimuli, occurring around 150-250 ms after stimulus onset. It represents cerebral responses to violations of regularities and has been shown in coma and DOC patients, as well as during anesthesia (Fischer et al., 1999; Heinke et al., 2004; Kotchoubey et al., 2005). Although MMN can discriminate DOC from healthy controls, it fails to discriminate UWS from MCS (Bekinschtein et al., 2009; Sitt et al., 2014). MMN is considered to reflect a pre-attentive, nonconscious response. Despite its limited capacity to discriminate levels of consciousness, MMN has been shown to predict patients' recovery of consciousness (Qin et al., 2008; Wijnen, van Boxtel, Eilander, & de Gelder, 2007).

The P300 is a positive component occurring 300-500 ms after the onset of deviant stimuli. It had been widely accepted as a reliable proxy for consciousness (Li, Song, Du, Huo, & Shan, 2015; Perrin et al., 2006); however, this notion has been challenged in the past decade (see a review paper by Forster et al., 2020). A more in-depth discussion of P300 is presented in section 2.2.4.

2.2.2.2 Speech paradigm

The N400 paradigm is a widely used speech paradigm, in which participants listen to sentences ending with either semantically congruent or incongruent words. The N400 is a negative component that occurs approximately 400 ms after incongruent words, indexing the detection of semantic violation. It has been observed in both MCS and UWS patients (Balconi, Arangio, & Guarnerio, 2013; Steppacher et al., 2013), without significant differences between groups (Beukema et al., 2016; Di Stefano, Cortesi, Masotti, Simoncini, & Piperno, 2012). This finding raises concerns about the diagnosis of UWS and the exact role of the N400 component in assessing consciousness.

On the one hand, the presence of N400 in UWS patients suggests that they may have preserved higher-level cognitive abilities related to semantic processing, challenging the traditional definition of UWS (i.e., complete unawareness). On the other hand, N400 may not be a definitive neurophysiological indicator of consciousness; instead, it might represent automatic processing. Supporting evidence reveals that the N400 occurs without conscious awareness, e.g., during sleep (Brualla, Romero, Serrano, & Valdizán, 1998; Ibanez, Lopez, & Cornejo, 2006). However, contradictory results were found in a study of dexmedetomidine-induced unresponsiveness, which reported a loss of N400 (Kallionpää et al., 2018). In contrast to the inconclusive role of the N400 in assessing remaining consciousness in DOC patients, the component has proven to be a more meaningful indicator of patient outcome prediction (Sokoliuk et al., 2021; Steppacher et al., 2013).

Additionally, synthesized speech including isochronous speech stimuli allows for the distinction of neural responses to various levels of linguistic processing. Specifically, speech sequences are constructed such that distinct linguistic levels (i.e., words, phrases, and sentences) correspond to distinct frequencies, enabling the identification of hierarchical speech processing based on neural responses at different frequencies (Ding, Melloni, Zhang, Tian, & Poeppel, 2016). Higher-level speech

tracking, such as sentence or phrase tracking, requires semantic understanding of the speech, and has recently been used to assess the depth of consciousness. For example, Makov et al. demonstrated that low-level auditory processing is preserved during sleep but higher-level hierarchical linguistic parsing is disrupted (Makov et al., 2017). Gui et al. reported that phrase-level tracking was preserved in the MCS group but not in the UWS group, while sentence-level tracking was impaired in both MCS or UWS groups. However, at the individual level, 11 out of 42 MCS patients and 4 out of 36 UWS patients showed significant sentence or phrase tracking, indicating residual consciousness in these patients (Gui et al., 2020). Given the limited number of studies investigating the use of this speech paradigm in DOC patients, we examined this aspect in both healthy participants and DOC patients in Chapters 2 and 4 of this thesis. This investigation contributes to a more comprehensive understanding of hierarchical speech processing and its potential role in assessing consciousness.

2.2.2.3 “Local-Global” paradigm

In the “local-global” paradigm, patients are presented with a series of sounds that contain hierarchical regularities at both local and global levels. The violation of the local regularity elicits a typical MMN response, which failed to discriminate UWS from MCS (Bekinschtein et al., 2009; Sitt et al., 2014). The implicit perception of global regularity violation is proposed as a signature of conscious processing (Bekinschtein et al., 2009; Faugeras et al., 2012; King et al., 2013). Although this paradigm has shown promise in assessing consciousness (Bekinschtein et al., 2009; Faugeras et al., 2012), it is worth noting that some studies have reported contradictory results. For example, a study by Sitt et al. indicated that the response to global regularity violation did not discriminate UWS from MCS patients (Sitt et al., 2014).

2.2.3 Active paradigms

Active paradigms involve the willful modulation of brain activity by participants in response to a given command (Monti et al., 2010). For example, Schnakers et al. presented DOC patients with a sequence of names containing patients’ own names (SON) and other names in both passive (passive listening) and active (instructing patients to count names) conditions (Schnakers, Perrin, et al., 2008). They observed a more prominent P300 in the active condition compared to the passive condition only in MCS but not UWS patients. This finding indicates the preservation of voluntary brain processing in MCS. Furthermore, motor imagery tasks, which analyze command-specific EEG responses, have demonstrated that many patients diagnosed with UWS can perform these tasks, suggesting that these patients might preserve residual consciousness that is difficult to detect through behavioral evaluations (Bruno et al., 2010; Cruse et al., 2011).

2.2.4 Near-threshold detection

Near-threshold detection is a commonly used paradigm in the studies of consciousness in healthy participants. In this paradigm, healthy participants are asked to report whether they are aware or unaware of a sensory stimulus. By comparing neural responses to aware vs. unaware identical stimuli while controlling for confounding variations in sensory input, neural markers of conscious perception can be identified. Results of previous EEG studies using this approach suggest that conscious perception involves two prominent ERP components: an early awareness-related negativity (ARN), in sensory regions 120-200 ms after the stimulus onset and a late positive component – P300, in occipital-parietal regions 250-500 ms after the stimulus onset (Dembski, Koch, & Pitts, 2021; Forster et al., 2020).

However, the precise roles of these components in consciousness remain a topic of ongoing debate. Recent research has challenged the notion that the P300 component represents

consciousness *per se*, proposing instead that it may represent the post-perceptual processing. This notion is supported by studies demonstrating that this component may not emerge in response to perceived yet unreported stimuli (stimuli in that study were irrelevant to the participants' task) (Cohen, Ortego, Kyroudis, & Pitts, 2020). Similarly, Schroder et al. reported that this component may not emerge from target detection when report requirements are controlled using a matching task (Schroder, Nierhaus, & Blankenburg, 2021). Along with this evidence, challenging the late component P300 as neural correlate of consciousness, a substantial body of research has provided strong support for the role of ARN in consciousness. However, this view is also challenged by the contamination of attention and further evidence is required to rule out the possibility that ARN is an indicator of attention (Bola & Doradzinska, 2021).

2.3 The hemodynamic approach using functional magnetic resonance imaging and positron emission tomography

Resting-state functional magnetic resonance imaging (fMRI) evaluates brain function at a resting state. Numerous studies have reported that the functional connectivity of the default mode network (DMN, including anterior and posterior midline regions) changes with the levels of consciousness (Boly et al., 2009; Demertzi et al., 2014). Moreover, other resting networks, such as the frontoparietal network, salience network, and auditory network have demonstrated high discriminative ability (>80%) for differentiating MCS and UWS patients (Demertzi et al., 2015).

During passive listening, Crone et al. found that the DMN deactivation was significantly reduced in DOC patients compared to healthy controls (Crone et al., 2011), suggesting that this deactivation could be a potential neuroimaging biomarker for assessing consciousness in DOC patients. Further studies have revealed activation in the primary auditory cortex and higher-order temporal areas in some UWS patients when listening to their own names, suggesting the presence of cognitive processing in these patients (Di et al., 2007).

A landmark study by Owen et al. utilized a task-based active paradigm and showed that a patient clinically diagnosed with UWS was able to follow commands by modulating brain activity during fMRI (Owen et al., 2006). This fMRI-based method has been used to complement behavioral assessment in DOC patients (Bardin et al., 2011; Braiman et al., 2018).

Positron emission tomography (PET) has also been used to detect consciousness. A recent study reported that fluorodeoxyglucose-PET achieved high sensitivity (93%) in differentiating UWS from MCS patients, and showed high congruence with behavioral CRS-R scores (85%) (Stender et al., 2014). The recent European Academy of Neurology (EAN) guidelines suggest that FDG-PET and fMRI (in resting state and during active paradigms) should be assessed as part of multimodal assessments (Kondziella et al., 2020).

In conclusion, these findings reveal an underestimation of consciousness in a subset of DOC patients who are diagnosed as UWS based on behavioral indicators, but classified as MCS based on neuroimaging indicators. According to the EAN guidelines, it is recommended to integrate clinical evaluation, EEG-based techniques and fMRI for a comprehensive multimodal evaluation of consciousness (Kondziella et al., 2020).

3 Review of consciousness restoration

3.1 Pharmacological treatments

Many pharmacological treatments, such as amantadine and zolpidem, have been investigated for their effectiveness in improving consciousness in DOC patients. In a large class-II randomized placebo-

controlled trial, amantadine, a dopaminergic drug, was administered for four weeks and was shown to accelerate functional recovery in DOC patients (Giacino et al., 2012). The positive effect was proposed to arise from neuromodulatory effects on the nigrostriatal, mesolimbic, and frontostriatal dopaminergic systems (Fridman & Schiff, 2014). In line with this finding, other studies reported amantadine to improve consciousness and increase metabolism in the frontoparietal cortex in MCS patients (Estraneo, Pascarella, Moretta, Loreto, & Trojano, 2015; Schnakers, Hustinx, et al., 2008). Because of these potential benefits, amantadine has been recommended as a promising treatment for promoting recovery in DOC patients (Thibaut, Schiff, Giacino, Laureys, & Gosseries, 2019).

Zolpidem, as a GABA-ergic medication, has been reported to induce temporary consciousness recovery in DOC patients. In a study by Thonnard et al., 60 patients with DOC were administered 10 mg of zolpidem, and the CRS-R was assessed one hour after administration. The diagnosis remained unchanged after zolpidem administration (Thonnard et al., 2013). However, in another placebo-controlled, double-blind, crossover study involving 84 patients, 4.8% of them were confirmed as responders, defined as experiencing clinical consciousness improvements lasting 1-2 hours after the administration of 10 mg of zolpidem (Whyte et al., 2014). Overall, studies using zolpidem have yielded conflicting results.

Recently, psychedelics have been proposed as a promising pharmacological option to treat DOC due to the potential of psychedelics to increase brain complexity, which has been reliably associated with increased levels of consciousness (Scott & Carhart-Harris, 2019; Timmermann et al., 2023). However, more empirical evidence is required to evaluate the value of psychedelics for treating DOC patients.

3.2 Invasive stimulation

Deep brain stimulation (DBS) is an invasive intervention that delivers electrical pulses to specific brain regions via surgically implanted electrodes. In 2007, Schiff and colleagues reported a case in which DBS targeted at the central thalamus led to behavioral improvements in a MCS patient (Schiff et al., 2007). Subsequently, several clinical trials have further evidenced the efficiency of DBS in modulating consciousness levels in DOC patients, highlighting its potential as a therapeutic method (Chudy et al., 2018; Yamamoto et al., 2010; Yang et al., 2023).

3.3 Sensory stimulation

Auditory stimulation is probably the most commonly used sensory stimulation method for promoting consciousness restoration. A study reported that after six weeks of familiar auditory stimulation, consisting of stories narrated by patients' relatives (10 min, four times per day), the DOC patients showed enhanced behavioral and neural responses (Pape et al., 2015). Moreover, previous studies have reported that music stimulation can evoke brain activation, increase brain connectivity and modulate physiological indices (Castro et al., 2015; Heine et al., 2015; Lancioni, Singh, O'Reilly, Sigafoos, & Desideri, 2021; Okumura et al., 2014). Specifically, Heine et al. reported that five DOC patients exhibited stronger functional connectivity between the left precentral gyrus and the left dorsolateral prefrontal cortex during music compared to a control condition (repetitive noise) (Heine et al., 2015). Additionally, music therapy has been observed to have positive effects on behaviors such as eye contact, smiles and voice (Raglio et al., 2014). These observations have indicated that music therapy can prompt the activation of related brain regions, promote the recovery of patients' behaviors and increase the level of consciousness in DOC (Yekefallah, Namdar, Azimian, Dost Mohammadi, & Mafi, 2021).

Visual stimulation, such as the presentation of familiar faces, has been shown to evoke brain activation in patients with DOC (Owen et al., 2002; Pan et al., 2014). However, the feasibility of visual

stimulation is limited due to the inability of DOC patients to maintain a fixed gaze. Vibrotactile stimulation has been explored less and requires further research. Only Cheng et al. found that sensory stimulation, including auditory, visual, tactile, olfactory, and gustatory modalities, led to improved consciousness in DOC patients (Cheng et al., 2018).

3.4 Non-invasive electromagnetic stimulation

3.4.1 Transcranial direct current stimulation

Transcranial direct current stimulation (tDCS) is a non-invasive brain stimulation technique that applies a low direct current to the scalp to modulate cortical excitability. In recent years, tDCS has been used to enhance consciousness in patients with DOC by targeting various brain regions, including the dorsolateral prefrontal cortex (DLPFC), motor cortex, orbitofrontal cortex, and posterior parietal cortex.

Single-session tDCS has shown limited effects on clinical assessments of consciousness, with one notable exception. A study evaluated the effects of 20 min of 1 mA anodal tDCS over the DLPFC on 55 DOC patients and found that 43% of MCS patients and 8% of UWS patients showed clinical improvements as measured by CRS-R (Thibaut, Bruno, Ledoux, Demertzi, & Laureys, 2014). Although many subsequent studies have reported that single-session tDCS increased cortical excitability, they found no significant changes in CRS-R scores (Aloi, Della Rocchetta, Ditchfield, Coulborn, & Fernández-Espejo, 2021; Bai et al., 2018; Martens et al., 2019). For example, Naro et al. applied single-session tDCS over the orbitofrontal cortex in 25 DOC patients and 20 healthy individuals and reported enhanced cortical connectivity and excitability in MCS patients and healthy controls, but no significant change in CRS-R scores (Naro, Calabro, et al., 2015). However, in a follow-up study, these researchers investigated the effects of 10 min of cerebellar oscillatory tDCS on cortical connectivity in patients with DOC (Naro, Russo, et al., 2016). They found that oscillatory tDCS induced changes in cortical connectivity and improvements in CRS-R scores (motor and arousal sub-scales) in MCS patients only, suggesting the potential of oscillatory tDCS for modulating brain connectivity and improving consciousness in patients with DOC.

When applied over multiple sessions, tDCS has been observed to improve consciousness, particularly in MCS patients (Angelakis et al., 2014; Thibaut et al., 2017). For instance, Thibaut et al. (2017) reported clinical improvements in nine out of 16 MCS patients after five daily sessions of tDCS over the left prefrontal cortex, with the treatment effect lasting for one-week post-stimulation (Thibaut et al., 2017). Nevertheless, a few studies have reported no significant treatment effects following multiple sessions of tDCS (Cavinato et al., 2019; Estraneo et al., 2017; Wu et al., 2019).

Overall, current research suggests that tDCS may lead to clinical improvements in patients in MCS, especially when administered over multiple sessions.

3.4.2 Transcranial alternating current stimulation

Compared to tDCS, which is a relatively basic approach modulating general cortical excitability, *transcranial alternating current stimulation* (tACS) provides a more targeted method for modulating brain oscillations at specific frequencies. In the context of DOC patients, only one study has investigated the effects of tACS in 26 DOC patients. This study applied gamma tACS over the right DLPFC for 10 min and recorded EEG before and after stimulation. The results revealed that gamma-band (ranging from 35 to 140 Hz) tACS specifically modulated large-scale cortical connectivity and excitability in all MCS patients and some UWS patients (Naro, Bramanti, Leo, Russo, & Calabro, 2016). However, no significant effects on CRS-R scores were observed.

A few studies involving healthy participants have reported the effects of tACS in modulating conscious perception in a phase-dependent manner (Gundlach, Muller, Nierhaus, Villringer, & Sehm,

2016; Neuling, Rach, Wagner, Wolters, & Herrmann, 2012; Riecke, Formisano, Herrmann, & Sack, 2015). In particular, considering the potential link between gamma oscillations and consciousness (Ghazanfar & Schroeder, 2006; Stein, Stanford, & Rowland, 2009), tACS has been applied at gamma frequencies to modulate consciousness. For example, Voss et al. applied tACS at different frequencies during rapid eye movement sleep and found that both 25 Hz and 40 Hz tACS increased ongoing gamma activity and induced self-awareness in dreams (Voss et al., 2014).

In summary, tACS offers a targeted approach for modulating brain oscillations at specific frequencies. Although the potential of gamma-band tACS for consciousness modulation has been demonstrated, the evidence remains limited. To provide more evidence, I explored the effects of electrical stimulation at gamma frequency in both healthy participants and DOC patients in Chapters 3 and 4. This investigation aims to better understand the potential of gamma-band stimulation for modulating oscillatory brain activity and enhancing consciousness.

3.4.3 Transcranial magnetic stimulation

Transcranial magnetic stimulation (TMS) is a non-invasive brain stimulation technique that employs a rapidly varying electromagnetic field to induce electrical currents in the brain that can depolarize neurons, evoke responses or alter neuronal plasticity (Hallett, 2000). Repetitive TMS (rTMS) involves applying repeated stimuli to specific cortical regions, resulting in either excitatory or inhibitory effects depending on the stimulation frequency. For consciousness improvement, rTMS typically involves stimulation frequencies of 10 Hz or 20 Hz, applied over the left DLPFC and primary motor cortex (M1).

However, single-session stimulation has been shown to be insufficient for enhancing consciousness (Bai et al., 2016; Liu et al., 2016; Naro, Russo, et al., 2015). In a study conducted by Bai et al., the CRS-R demonstrated changes after nine consecutive sessions of rTMS. Nonetheless, studies on the effects of multiple rTMS sessions on DOC are often underpowered and yield mixed results. While some studies suggest that 10 or 20 sessions of rTMS may increase behavioral assessments of consciousness, serum estradiol levels, and brain responses (Fan, Zhong, Wang, Aierken, & He, 2022; He et al., 2021; Xia et al., 2017), two other studies report that five sessions of rTMS produced no effects or only minor effects on DOC (Cincotta et al., 2015; He et al., 2018). These findings indicate that an adequate number of stimulation sessions is crucial for inducing the effectiveness of rTMS.

3.4.4 Transcutaneous stimulation

The effectiveness of transcranial current stimulation is currently debated based on findings showing that cortical effects may be an epiphenomenon of co-stimulation of peripheral nerves (Asamoah, Khatoun, & Mc Laughlin, 2019). Considering these concerns, a more effective non-invasive approach to modulate neural activities may be transcutaneous stimulation (Adair et al., 2020; Conlon et al., 2020). This approach involves the application of an electrical current to the skin directly above a peripheral or cranial nerve. Currently, various forms of transcutaneous stimulation have been investigated for their potential in treating DOC.

Transcutaneous auricular vagal nerve stimulation (taVNS) is an emerging transcutaneous stimulation technique that targets the tenth cranial nerve, the vagus nerve. Stimulation of the auricular branch activates the spinal trigeminal nucleus and the nucleus solitarius, subsequently leading to the activation of the thalamus and cortical areas (Jang & Cho, 2022). The first case reported by Yu et al. (Yu et al., 2017) demonstrated a patient with UWS transitioning to MCS after four weeks of taVNS treatment, as the CRS-R score improved from 6 to 12. Meanwhile, the fMRI results revealed increased functional connectivity in the DMN after treatment. To date, seven studies have investigated the efficiency of taVNS in improving consciousness, with common stimulation parameters including 20 or 25 Hz pulsed stimulation applied to the left or bilateral auricular concha for multiple

sessions (e.g., 4 or 8 weeks, 14 days, or 6 months). These studies have been shown to increase CRS-R scores, decrease delta and increase beta EEG power (Yifei et al., 2022), and enhance cerebral blood flow (Yu et al., 2021). However, only a recent study by Zhou et al. (Zhou, Kang, Xiong, Feng, & Dong, 2023) included a sham stimulation group to control for the effects of placebo and/or spontaneous recovery. While no significant CRS-R changes were observed in the overall group, MCS patients were found to exhibit significant improvements in consciousness after 4-week daily stimulation (Zhou et al., 2023).

In addition to taVNS, *median nerve stimulation* (MNS) has been shown to excite the brainstem reticular system and the cerebral cortex. Research has shown that MNS increased the GCS score, decreased the days spent in the intensive care unit, and induced a high proportion of patients who gained consciousness after coma (Cooper, Jane, Alves, & Cooper, 1999; Lei, Wang, Gao, Cooper, & Jiang, 2015; Wang et al., 2022). However, as MNS is more commonly used in the acute state of consciousness disorders (i.e., coma), studies on chronic DOC are lacking.

Trigeminal nerve stimulation (TNS) is a novel stimulation approach for DOC. Evidence from animal models showed that TNS in rats with impaired consciousness can upregulate neuropeptide hypocretin in the lateral hypothalamus and this activation can promote consciousness recovery (Zheng et al., 2021). In DOC patients, two studies reported improvements in consciousness after TNS (Dong, Tang, Fang, & Feng, 2022; Fan et al., 2019). Thus, both animal and human findings suggest that TNS may be an effective approach for promoting consciousness.

Despite the potential demonstrated by many aforementioned treatments (Fig. 1), there remains a lack of universally recognized and established therapeutic approaches for patients with DOC. In my thesis, I have conceptualized a neuroscience-based method that integrates multimodal stimulation. This novel approach is detailed in Section 4.

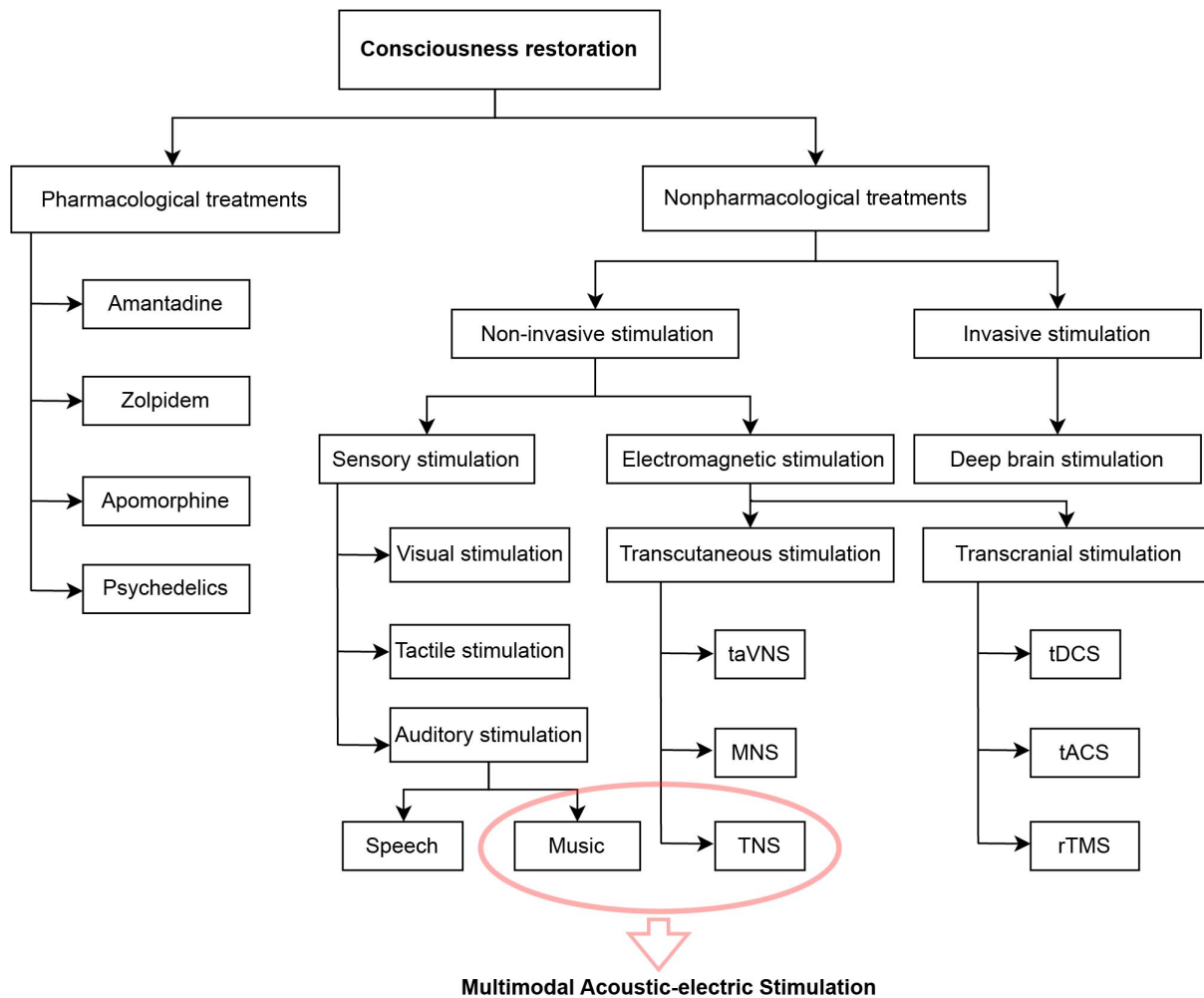


Figure 1. Overview of treatment methods for consciousness restoration.

4 Rhythmic acoustic-electric trigeminal nerve stimulation

4.1 Trigeminal nerve

The trigeminal nerve, the fifth and most complex cranial nerve, consists of three branches: the ophthalmic nerve (V1), the maxillary nerve (V2), and the mandibular nerve (V3) (Fig. 2). The ophthalmic nerve (V1), which is purely sensory, supplies the forehead, upper eyelid, cornea, conjunctiva, dorsum of the nose, and dura of some of the anterior cranial fossa. The maxillary nerve (V2), also purely sensory, supplies the region below the orbits and above the mouth. The mandibular nerve (V3) has both sensory and motor components, carrying sensory information from the lower lip, lower teeth and gums, chin and jaw, parts of the external ear, and parts of the meninges, as well as controlling the muscles of mastication.

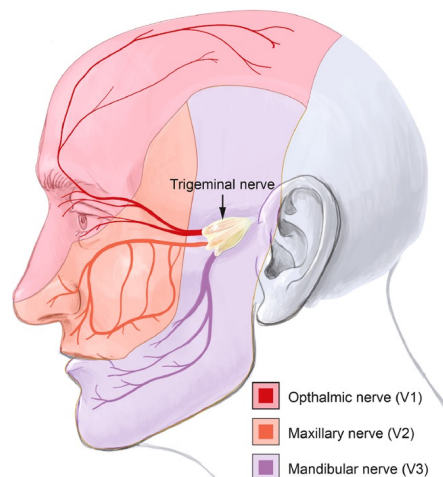


Figure 2. Illustration of the three branches of the trigeminal nerve (ophthalmic, maxillary, and mandibular) and their corresponding facial territories. Adapted from Ferneini, 2021.

The trigeminal nerve conveys sensory information, including touch, pain, and temperature, which is relayed through three trigeminal nuclei in the brainstem before traveling to the thalamus and ultimately projecting to the somatosensory cortex. These nuclei comprise the spinal tract nucleus, which processes pain and temperature; the main sensory nucleus, which processes tactile sensation; and the mesencephalic nucleus, which processes proprioceptive sensibility. Furthermore, these trigeminal nuclei activate neurotransmitter pathways, such as the locus coeruleus (norepinephrine pathway) and the raphe nuclei (serotonin pathway). Both are components of the reticular activating system and are thus considered as relevant nuclei for consciousness (Khrouf, Reddy, & Saadabadi, 2023).

Trigeminal nerve stimulation has been considered as a safe and well-tolerated treatment for various disorders, including migraine, depression, epilepsy and traumatic brain injury (Cook, Espinoza, & Leuchter, 2014; DeGiorgio et al., 2013; McGough et al., 2019). The first application of TNS was for trigeminal neuralgia in the 1960s, when Wall and Sweet stimulated the trigeminal nerve (infraorbital nerve) by inserting an electrode at the infraorbital foramen and found a suppressed pain perception (Wall & Sweet, 1967). The use of TNS to promote consciousness is still relatively novel, with the first application in human participants reported in 2019 by Fan and colleagues (Fan et al., 2019). They applied 40 Hz TNS with an intensity of 18-20 mA continuously for 6 hours per day for a total of 6 weeks in a single DOC patient and reported an improvement in consciousness after the TNS. Another study by Dong et al. (Dong et al., 2022) investigated the effects of TNS in 21 DOC patients and reported that eight out of them demonstrated improved CRS-R scores. However, these two studies did not include sham control groups, leaving the potential effects of placebo or other confounding factors uncertain. In addition to human evidence, animal studies have shown that TNS can upregulate neuropeptide hypocretin, protect the blood-brain barrier and reduce the level of brain edema (Chiluwal et al., 2017; Yang et al., 2022; Zheng et al., 2021).

In conclusion, based on the neural anatomy of the trigeminal nerve and existing findings on TNS, stimulating the trigeminal nerve to improve consciousness appears to be a meaningful and promising approach.

4.2 Multimodal stimulation

Multimodal stimulation has shown promise in restoring brain function and treating neurological diseases, such as tinnitus and Alzheimer's disease (AD), by demonstrating stronger behavioral and neural effects than unimodal stimulation. The unique enhanced effects of multimodal stimulation can be attributed to the synergistic effects of simultaneous activation of multiple sensory pathways (Ghazanfar & Schroeder, 2006; Stein et al., 2009).

In tinnitus, multimodal acoustic-electric stimulation has been found to modulate physiological responses (i.e., neural firing rates) and behavioral performances (i.e., tinnitus perception) in a guinea pig model (Marks et al., 2018). These findings were further corroborated in human participants, where bimodal acoustic-electric stimulation, but not unimodal acoustic stimulation, reduced tinnitus symptoms (Marks et al., 2018).

In AD, a combination of 40 Hz auditory tone stimulation with 40 Hz light flicker has demonstrated a larger effect on brain oscillations and reduction of amyloid load—a pathological hallmark of AD—than either sensory modality alone (Martorell et al., 2019). This synergistic effect may be due to the recruitment of additional neural circuits that are involved in multisensory processing, leading to a more efficient processing and targeted modulation of brain function (Driver & Noesselt, 2008).

Multimodal stimulation (including auditory, visual, tactile, olfactory, and gustatory stimulation) has also been used to restore consciousness; however, in these cases, each sense was stimulated sequentially rather than simultaneously (Cheng et al., 2018; Megha, Harpreet, & Nayeem, 2013), precluding multisensory integration. Despite the potential benefits of multimodal stimulation for consciousness recovery, the current evidence supporting its effectiveness remains limited. Therefore, a primary focus of this thesis is to investigate the effects of multimodal stimulation on consciousness.

Acoustic musical stimulation, due to its convenience and promise for consciousness restoration (as discussed in Section 3.2), is selected as one component of the multimodal stimulation. The other modality incorporated is electric-tactile trigeminal nerve stimulation, as introduced earlier (Section 4.1). Thus, this multimodal stimulation approach combines musical stimulation and electric-tactile trigeminal nerve stimulation. Furthermore, considering the evidence presented in Sections 1 and 3.3.2, which supports the association between gamma oscillations and consciousness, the multimodal stimulation is applied at gamma frequency with the aim of modulating gamma activity. The comprehensive effectiveness of gamma-band acoustic-electric stimulation on consciousness, oscillatory brain activity, and brain connectivity is investigated in Chapters 3-5.

5 Outline of the thesis

This dissertation developed a novel approach that combines multimodal stimulation, integrating both acoustic musical stimulation and electrical trigeminal nerve stimulation, modulated at gamma frequency. I investigated the potential effects of this multimodal stimulation approach on consciousness restoration in both healthy individuals (Chapter 3) and patients with DOC (Chapter 4). In order to provide an objective assessment of consciousness, I first developed an EEG-based speech paradigm that requires semantic processing and evaluated its usefulness in healthy participants (Chapter 2). Finally, I examined the impact of the multimodal stimulation on functional brain connectivity (Chapter 5). In sum, this thesis explored the potential benefit of rhythmic acoustic-electric stimulation combining musical stimulation and electrical trigeminal nerve stimulation on consciousness and its underlying neurophysiological mechanisms.

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Chapter 2

Sentential contextual facilitation of auditory word processing builds up during sentence tracking

Based on

Wu, M., Bosker, H.R., Riecke, L. (2023). Sentential contextual facilitation of auditory word processing builds up during sentence tracking. *Journal of Cognitive Neuroscience*, 35, 1262-1278

Abstract

While listening to meaningful speech, auditory input is processed more rapidly near the end (vs. beginning) of sentences. Although several studies have shown such word-to-word changes in auditory input processing, it is still unclear from which processing level these word-to-word dynamics originate. We investigated whether predictions derived from sentential context can result in auditory word-processing dynamics during sentence tracking. We presented healthy human participants with auditory stimuli consisting of word sequences, arranged into either predictable (coherent sentences) or less predictable (unstructured, random word sequences) 42-Hz amplitude-modulated speech, and a continuous 25-Hz amplitude-modulated distractor tone. We recorded reaction times (RTs) and frequency-tagged neuroelectric responses (auditory steady-state responses, ASSRs) to individual words at multiple temporal positions within the sentences, and quantified sentential context effects at each position while controlling for individual word characteristics (i.e., phonetics, frequency, and familiarity). We found that sentential context increasingly facilitates auditory word processing as evidenced by accelerated RTs and increased ASSRs to later-occurring words within sentences. These purely top-down contextually driven auditory word-processing dynamics occurred only when listeners focused their attention on the speech and did not transfer to the auditory processing of the concurrent distractor tone. These findings indicate that auditory word-processing dynamics during sentence tracking can originate from sentential predictions. The predictions depend on the listeners' attention to the speech, and affect only the processing of the parsed speech, not that of concurrently presented auditory streams.

Keywords

Word processing, sentential prediction, context effects, frequency tagging

1 Introduction

Speech comprehension is crucial for social communication in everyday life. It is thought to emerge from multiple auditory and linguistic processes including the identification of individual words in auditory input and the integration of words into syntactic and semantic structure (Ding, Melloni, Zhang, Tian, & Poeppel, 2016; Friederici, 2002; MacDonald, Pearlmutter, & Seidenberg, 1994). Besides such contributions from auditory word processing to the formation of higher-level syntactic and semantic information, a contribution in the opposite direction—i.e., a top-down effect of syntactic and semantic processing on lower-level auditory word processing—also exists (Davis, Ford, Kherif, & Johnsrude, 2011; Friederici, 2012; Guediche, Reilly, Santiago, Laurent, & Blumstein, 2016; Kaufeld et al., 2020; Vigliocco et al., 2007). A striking illustration is temporal position effects in unambiguous speech, where auditory processing evolves incrementally as the meaning of the ongoing speech unfolds. For example, detection of brief acoustic events (e.g., clicks) has been shown to be quicker when these events occur during words late (vs. early) in a sentence (Holmes & Forster, 1970; Lobina, Demestre, & Garcia-Albea, 2018). This facilitation of auditory processing across word positions has also been observed in linguistic tasks requiring detection of target words in syntactically and/or semantically intact sentences (Marslen-Wilson & Tyler, 1975; Oliver, Gullberg, Hellwig, Mitterer, & Indefrey, 2012). These behavioral benefits may be enabled by corresponding across-word increases in neural oscillations in the gamma and theta bands, as suggested by neurophysiological studies on written word processing (Fedorenko et al., 2016; Lam, Schoffelen, Udden, Hulten, & Hagoort, 2016). In sum, the processing of acoustic events and words accelerates in a word-by-word manner during sentence processing, here referred to as “word-position effect”.

A common interpretation of the word-position effect on auditory processing is that it originates from top-down processes involving semantic and syntactic predictions. The grouping of words into phrases can create semantic context from which the meaning of upcoming words can be predicted (Kuperberg & Jaeger, 2016; Staub, 2015). This semantic prediction has been demonstrated in many neurophysiological studies showing an enlarged N400 scalp potential evoked by unexpected words compared to expected words (Grisoni, Tomasello, & Pulvermüller, 2021; Kutas & Federmeier, 2011). Apart from semantic prediction, listeners may also employ syntactic prediction to anticipate the syntactic properties of upcoming words in grammatical sentences (Demberg, Keller, & Koller, 2013; Ferreira & Qiu, 2021). Based on the anticipation of meaning of upcoming words, the processing of later-occurring words in a sentence may be facilitated. A potential factor for this facilitation may be top-down attention: even shorter reaction times to later words have been observed when listeners are semantically primed to focus their attention on the later-occurring words in sentences (Cutler & Fodor, 1979). However, the precise role of attention in the word-position effect remains to be determined.

Another interpretation of the word-position effect puts more emphasis on auditory predictions derived from the overt prosody carried by the acoustic signal (Foltz, 2020; Ito & Speer, 2008). This view is supported by behavioral findings demonstrating the word-position effect within pseudo-sentences that contain no valid syntax or meaning but normal intonation (Tyler & Warren, 1987). However, interpretation of these previous observations in terms of top-down predictions is hampered by the fact that words at different positions differed not only in their lexical meaning and/or intonation, but also other individual word characteristics such as their phonetic detail, frequency of occurrence, and familiarity. Thus, it is still unclear from which processing level these dynamics in auditory processing originate. According to the current body of evidence, they may be driven by variations in word meaning, word phonetics, word frequency, word familiarity, or any of their combinations.

The present study aimed to disambiguate these possibilities. To this end, it tested the hypothesis that the dynamics in auditory processing during sentence processing can be driven

exclusively by top-down factors, in particular sentential predictions derived from the speech². Under our hypothesis, the word-position effect should (i) be observable during sentence processing even when individual word characteristics are held constant, (ii) depend on the listener's focus of endogenous top-down attention, and (iii) affect selectively the processing of the speech stream, i.e., it should not transfer to the processing of a concurrent auditory stream.

We presented participants with either semantically coherent sentences or acoustically-matched word sequences. In order to evaluate participants' ability to track sentence structure and to rule out potentially confounding prosodic cues, we used artificial isochronous speech (Ding et al., 2016). We recorded responses to individual words at multiple positions within the sentences. From these responses within sentential context, we subtracted responses to the acoustically-identical words in randomly ordered word sequences to measure *sentential contextual facilitation* (referred to as "SCF") at each position. To test our second and third predictions, we directed participants' attention to either the speech or a simultaneously ongoing distractor tone and simultaneously assessed cortical responses to speech or tone using a frequency-tagging technique. Previous neural studies of auditory speech processing have used frequency tagging to, for example, disentangle unisensory brain responses to simultaneously presented audiovisual speech (Drijvers, Jensen, & Spaak, 2021; Giani et al., 2012) or assess the internal integration of speech units (Ding et al., 2016; Ding et al., 2018) and implicit statistical learning (Batterink & Paller, 2017; Pinto, Prior, & Zion Golumbic, 2022; Zhang, Riecke, & Bonte, 2021). Frequency tagging involves the periodic modulation of a given stimulus feature at a specific frequency ("tagging frequency"), which results in brain activity that is phase-locked to the modulation and observable in neural recordings as a spectral peak at the tagging frequency.

We predicted that (i) SCF varies systematically across word positions, and that this putative word-position effect is modulated by (ii) the variation in the participants' focus of attention, and (iii) does not affect the processing of the simultaneous distractor tone.

2 Methods

2.1 Participants

Twenty-eight fluent speakers of English (20 females, mean age = 23.8 years, age range = 19-44 years) participated in the experiment. The number of participants was chosen based on previous studies (Ding et al., 2018; Oliver et al., 2012). To ensure that all participants were fluent speakers of English, we included only native English speakers (N = 3) and Maastricht University students enrolled in programs for which formal proof of English proficiency (CEFR level C1 or a similar accredited certification) is an admission requirement. All participants reported normal hearing and no history of neurological or psychiatric disorders. Written informed consent was obtained prior to the experiment. Participants were compensated with study credits or monetary reward for their participation. The experimental procedure was approved by the local research ethics committee (*Ethical Review Committee Psychology and Neuroscience, Maastricht University*).

2.2 Stimuli

2.2.1 Speech stimuli

Auditory word processing was elicited by presenting auditory stimuli consisting of 86 unique semantically valid four-word English sentences. Half of the sentences were composed of the syntactic

² We chose to frame the present study in terms of prediction without taking a particular stance in the debate between prediction and integration (Pickering & Gambi, 2018).

structure [noun—verb—adjective—noun] (e.g., *Cats eat fresh fish*) whereas the other half had the syntactic structure [adjective—adjective—noun—verb] (e.g., *Two big dogs bark*); these two syntactic structures are respectively referred to as NVAN and AANV for simplicity. While both structures contained a noun phrase and a verb phrase, the two structures differed strongly in the order and complexity of these phrases and words of the same category (noun, adjective, or verb) never occurred at the same position. Each trial consisted of eight different consecutive sentences with a fixed, predictable syntactic structure. The first sentence was the same in all trials of a given syntactic structure and served as a preparation of the participants for the upcoming speech syntax (this preparatory sentence was excluded from data analysis). The remaining 84 unique sentences (86 minus the two syntax-specific preparatory sentences) were distributed randomly across 12 consecutive trials. This set of trials was presented six times within each condition, each time in a different random order. No word was immediately repeated. All words were monosyllabic and synthesized independently using text-to-speech software in Mac OS (male voice USA Alex) to avoid systematic overt prosody differences across words. Every word spanned an interval of 360 ms; this was achieved by applying time compression with a stretch factor ranging from 0.49 to 0.92 to the original sound waveform (speech acoustics are presented in the appendix). Values of stretch factor below one represent compression; thus, only compression, but no expansion, was applied to the words. The speech stimuli were presented monaurally to participants' right ear (Fig. 1A).

2.2.2 Acoustic control stimuli

To allow ruling out confounding effects of word phonetics, frequency, or familiarity, appropriate control speech stimuli were included. These stimuli contained semantically anomalous versions of the 86 sentences and were constructed by shuffling all words across sentences (separately for each syntactic structure) except for target words (for details see section *Task*), which were always kept at their original positions (Fig. 1B). The resulting random word sequences (or “unstructured speech”) contained less predictable syntactic or semantic structure and they were verified that no structured sentence was accidentally created. Analogous to the structured speech stimuli, the first four words were the same in all trials and no word was immediately repeated.

2.2.3 Distractor tone

To allow investigating effects of endogenous selective attention on speech processing (for details see below, section *Task*) and a word-position effect on a concurrent auditory stream, an auditory distractor stimulus was presented simultaneously with the speech stimuli. The distractor was an ongoing 250-Hz pure tone presented monaurally to participants' left ear, contralateral to the speech. The choice for a tonal distractor was motivated by previous studies showing sentential context effects on the processing of nonlinguistic auditory stimuli such as clicks (reviewed in Introduction) and environmental sounds (Uddin, Heald, Van Hedger, Klos, & Nusbaum, 2018).

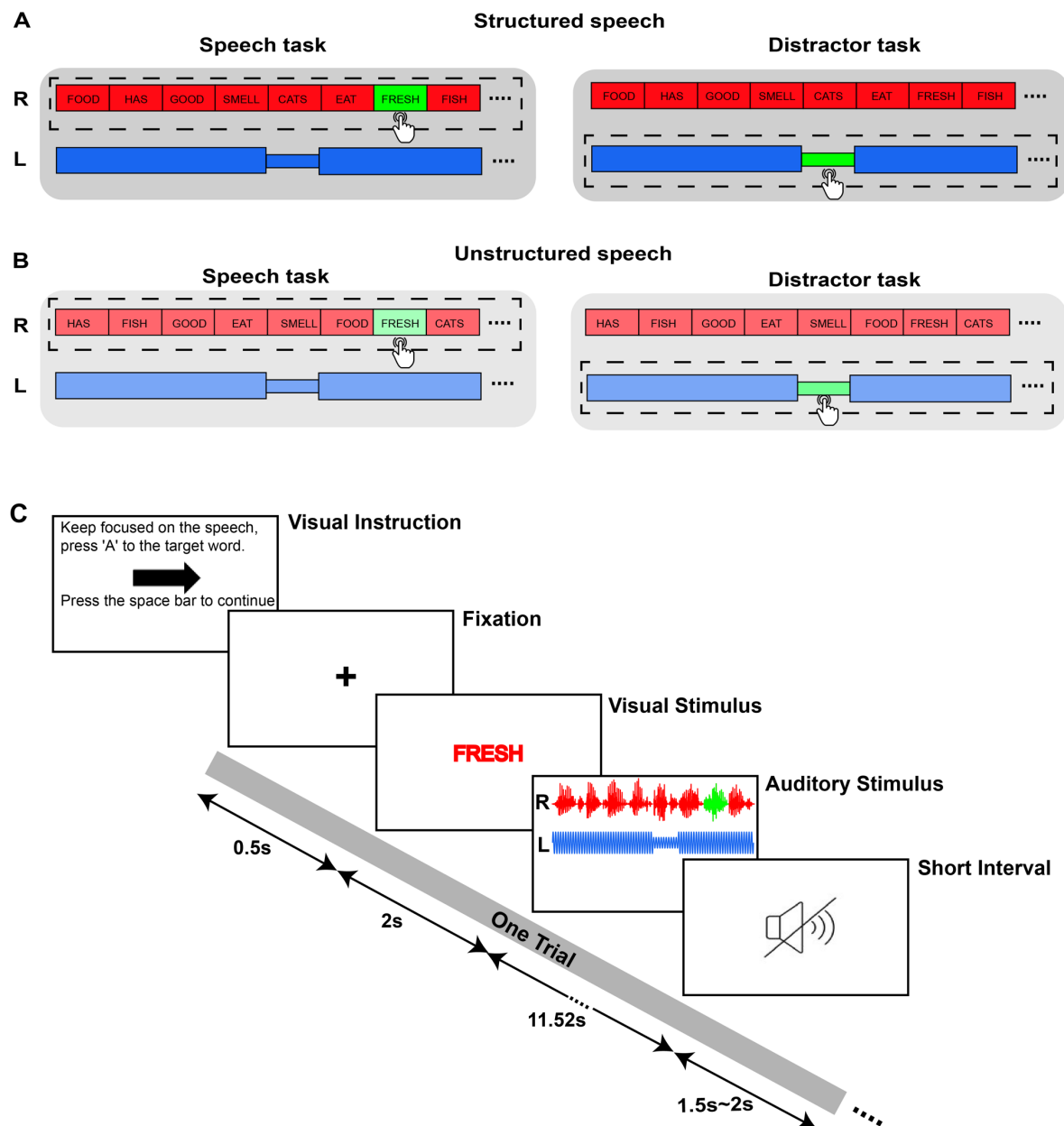


Figure 1. Auditory stimuli, experimental design, and trial design. (A). Illustration of the two main experimental conditions (gray boxes) involving structured speech. Sequences of red rectangles represent the speech stream (in the right ear, R) and sequences of blue rectangles represent the simultaneously presented distractor tone (in the left ear, L). Targets are depicted in green. The left panel shows conditions involving the speech task, the right panel illustrates the distractor task in which acoustically-matched stimuli were presented. Large dashed contours indicate participants' focus of selective attention. (B). Same as (A), but for the control condition. The control condition was identical to the main experimental condition, except that word order was shuffled, which rendered the continuous speech less predictable. (C). Schematic of an exemplary trial. First, a task instruction was presented on the screen, followed by a fixation cross at the center of the screen. Next, a word (in this example: "fresh") was shown, starting 2 s before the onset of the auditory stimulation (speech stream and distractor tone); this word was treated as a target only in the speech task. Participants had to give a response as soon as they heard the target (in this example the word "fresh"). Consecutive trials were separated by silent intervals lasting between 1.5 s-2 s.

2.2.4 Frequency tagging

To allow assessing separately the auditory processing of the speech and the distractor, a frequency-tagging technique was used. Amplitude modulations (sinusoidal waveform, modulation depth: 100%, fixed phase at the start of each word interval) with different rates were applied to the speech stimuli ($41 + \frac{2}{3}$ Hz, hereafter called 42 Hz for simplicity) and the distractor (25 Hz). These modulation rates have been shown to evoke strong phase-locked auditory cortical responses (Gransier, van Wieringen, & Wouters, 2017; Ross, Borgmann, Draganova, Roberts, & Pantev, 2000; Schoonhoven, Boden, Verbunt, & De Munck, 2003). As the eight four-word sentences within each trial were presented continuously at a fixed rate of $(4 \times 360 \text{ ms})^{-1}$ (i.e., 0.69 Hz), neural responses at this rate tagged participants' detection of the sentence structure.

2.3 Tasks

To allow assessing effects of endogenous selective attention, participants' attention was experimentally drawn toward the speech stimuli, or away from them, using two detection tasks with acoustically-matched stimulation (Fig. 1A). In the word-detection task (hereafter called "speech task"), the target was a specific word embedded in the continuous auditory speech. To obtain a measure of auditory word processing at the linguistic level, the target word was defined to participants through the visual sensory modality (Fig. 1C), which we deemed to encourage linguistic processing in the auditory speech task. In the tone-loudness change detection task (hereafter called "distractor task"), the target was a temporary loudness decrease (-5.1 dB) in the ongoing tone (target duration: 360 ms including 10-ms on/off ramps, corresponding to a single word interval).

In each task, participants were visually instructed to pay attention to the relevant auditory stream (speech or distractor tone), ignore the other stream, and detect and report the target in the attended stream as quickly as possible by pressing a key with the index finger of their right hand. Based on previous related studies (Jin, Zou, Zhou, & Ding, 2018; Oliver et al., 2012), only keypresses that fell into a 1440-ms window (corresponding to four consecutive word intervals) starting from the onset of the target were analyzed. Limiting the data analysis to responses within shorter windows (e.g., 720 ms, corresponding to two word intervals) did not alter the results qualitatively. In each task, targets occurred between one to three times per trial with approximately equal probability at each word position. Targets never occurred within the first sentence of a trial. As mentioned above, that sentence was presented only to inform participants of the upcoming speech syntax and never analyzed. Targets from different tasks (i.e., a given target word and a tone-loudness decrease) never concurred during the same interval.

2.4 Procedure

The experimental procedure involved the following steps: first, prior to the experimental session, participants were screened for hearing impairments and neurological or psychiatric disorders using a questionnaire. Second, participants were seated in a sound-attenuated and electrically shielded chamber. To reduce potential adaptation or learning effects in the main EEG experiment, participants were first familiarized with the stimuli and tasks during four practice blocks, each consisting of twelve trials, after which they received feedback on their performance. This practice was repeated until participants achieved a target detection accuracy of 80% or higher in each block. Four participants who failed to meet the criterion were excluded from the main experiment.

In the main experiment, four blocks, each containing 72 trials (i.e., 576 sentences) and lasting 18 min, were presented in individually randomized order while EEG was recorded. In one block,

participants performed the speech task on the structured speech stimuli (Fig. 1A left), which were presented in halves representing the two syntactic structures (presentation order was randomized individually). In another block, the same stimuli were presented, but participants performed the distractor task (Fig. 1A right). These two blocks represented the two main experimental conditions. The remaining two blocks were identical to the main ones above, except that the control stimuli (random word sequences) instead of the structured speech stimuli were presented (Fig. 1B).

Each block started with the presentation of a task instruction on the screen, followed by a central fixation cross and a word that remained visible during the subsequent auditory stimuli, which started 2 s later. Participants were instructed to pay attention to the visual word and treat it as a target only when they had received the instruction to perform the speech task. The inter-trial interval was randomized between 1.5 s to 2 s and no feedback on task performance was given. Participants could take a break after 36 trials (half a block, after which the syntactic structure switched) for as long as they needed. All stimuli were presented using Presentation software (Version 16.0, Neurobehavioral Systems, Inc) and insert earphones at ~69 dB_{SPL} (speech stimuli) and ~67 dB_{SPL} (distractor tone).

2.5 EEG recording

EEG was recorded using a 64-channel active BrainCap (Brain Products, Munich, Germany) in the standard 10–20 system. All EEG electrodes were referenced online to scalp position FCz. Electrode impedances were kept below 10k Ω . The EEG recordings were bandpass-filtered (cutoffs: 0.01 and 200 Hz, analog filter) and digitized with a sampling rate of 1000 Hz.

2.6 Data analysis

2.6.1 Behavioral data analysis

Behavioral performance was assessed based on response accuracy (percentage of correctly recognized targets) and the average reaction times (RTs) associated with correct responses.

2.6.2 EEG preprocessing

EEG data preprocessing was performed offline using EEGLAB 2019.1 (Delorme & Makeig, 2004) and MATLAB 9.4 as follows. First, bad channels with a leptokurtic voltage distribution (i.e., kurtosis higher than five) were replaced by interpolating between the surrounding channels (spherical spline interpolation; percentage of interpolated channels: 2.9 ± 1.8 , mean \pm SD across participants). Second, the interpolated channel data were re-referenced to an average reference. Third, independent component analysis was applied to the channel data to reduce artifacts. For this analysis the data were first 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 using the EEGLAB plugin ICLables (Pion-Tonachini, Kreutz-Delgado, & Makeig, 2019) and discarded (artifactual components: 15.8 ± 6.7 ; mean \pm SD across participants). The weights of the non-artifactual components were reapplied to the original unfiltered channel data (Jaeger, Bleichner, Bauer, Mirkovic, & Debener, 2018). Three participants were excluded from further analysis due to excessive artifactual components.

2.6.3 Analysis of frequency-tagged neural responses

To assess sentence- and word-rate responses, the continuous EEG data were segmented into 10.08-s epochs resembling single trials excluding the first sentence interval (which was discarded to avoid onset effects). Epochs were averaged across trials in the time domain separately for each condition

and then submitted to a discrete Fourier transform (DFT, 10080 points, resulting in a spectral resolution of approximately 0.1 Hz). The resulting spectra were averaged across all EEG channels.

Auditory steady-state responses (ASSRs) to the individual words and the distractor were assessed as described above for sentence- and word-rate responses, except for the two following differences: the 10.08-s epochs were further segmented into 360-ms epochs corresponding to word intervals, and consequently, a reduced number of DFT points was used, resulting in a spectral resolution of 2.78 Hz. To investigate cortical processes involved in sentence tracking, the data analysis was focused on scalp sites showing strong sentence-tracking responses. The strongest responses were found distributed mainly over the central-frontal scalp area (channels: AFz, Fz, F1, F2, F3 and FC1; Fig. 2A); thus, these EEG channels were selected and averaged in the frequency domain before statistical analysis.

2.6.4 Extraction of word-position effects

To allow assessing effects of word position on auditory processing, neural and behavioral responses were analyzed according to the position (1st, 2nd, 3rd, or 4th word interval) at which the stimulus or target occurred within the (pseudo-)sentences.

To obtain a measure of sentential contextual facilitation that is unrelated to word phonetics, frequency, or familiarity, the responses to the control speech stimuli were unshuffled, averaged across trials in the time domain (i.e., the constituent words of the unstructured speech were rearranged so that their order matched that of the original, semantically intact sentences), and subtracted from the responses to the matching intact sentences before statistical analysis. We refer to the resulting unbiased, acoustically-controlled behavioral and neural measure as “SCF” (sentential contextual facilitation, see *Introduction*). Note that within-trial positional differences between shuffled words and their unshuffled counterparts varied randomly across trials with a mean close to zero; therefore, the distance between shuffled and unshuffled words could not induce any systematic bias in SCF. Note further that we reversed the sign of the behavioral SCF; thus, positive SCF values represent accelerated RTs and negative values represent decelerated RTs.

The aforementioned analysis steps (stratification according to position and subtraction from unshuffled responses) were applied analogously to responses to the speech (RTs to target words and 42-Hz ASSRs during a given word interval) and to responses to the distractor (RTs to tone-loudness decreases and 25-Hz ASSRs during a given word interval).

2.6.5 Analysis of monotonicity

To assess monotonous increases of SCF across word positions, Sen’s slope (β) was calculated for each participant. Sen’s slope is a robust, nonparametric estimate of slope (for more details, see (Sen, 1968)) and is calculated as

$$\beta = \text{Median}\left(\frac{x_j - x_i}{j - i}\right)$$

where x_j and x_i are the data values at positions j and i respectively ($j > i$). Positive values of β indicate upward trends across positions.

2.7 Statistical analysis

The single-subject estimates from the 21 subjects were submitted to second-level (random-effect) statistical analyses. A significance criterion $\alpha = 0.05$ was used and type-I error probabilities inflated by multiple comparisons were corrected by false-discovery rate (FDR).

2.7.1 Spectral peak

Spectral peaks at tagging frequencies were compared to the average spectral response at the six neighboring frequency bins (noise floor, excluded the 50 Hz line noise) using a paired t-test. The statistical test was applied to each tagging frequency: 0.69 Hz (sentence rate), 1.39 Hz (second harmonic), 2.08 Hz (third harmonic), 2.78 Hz (word rate), 25 Hz (tone tag), and 42 Hz (speech tag). The spectral peaks at speech rates were further compared between the structured speech condition and control condition with a paired t-test.

2.7.2 Sentential contextual facilitation

Statistical analysis of the word-position effect involved a one-way repeated measures analysis of variance (ANOVA) including the factor "position" (word interval: 1st, 2nd, 3rd, or 4th) and two-way repeated-measures ANOVAs including an additional factor: either "attention" (speech task/attended vs. distractor task/unattended), or "sound stream" (speech stream [RTs to target words; 42-Hz ASSR] vs. tone stream [RTs to tone-loudness decreases; 25-Hz ASSR]). The two syntaxes were pooled. To assess the sentential contextual facilitation at each word position and its across-word trend, paired t-tests were applied that respectively compared SCF at each position to zero and Sen's slope to zero. The assumption of normality was verified with Kolmogorov-Smirnov tests, which did not detect any significant deviation from normality (all $p > .05$). The assumption of sphericity was assessed with Mauchly's tests and Greenhouse-Geisser correction was applied to adjust the degrees of freedom when the assumption was violated.

To further seek evidence of alternative hypotheses (H1) versus the null hypotheses (H0), Bayesian repeated-measures ANOVAs including the factor "position" and, where applicable, the additional factor "attention" or "sound stream" were carried out in JASP (Love et al., 2019). Evidence was inferred from the Bayes Factor (BF_{10}), defined as the ratio of the likelihood of the data fitting the alternative hypothesis to the likelihood of the data fitting the null hypothesis. BF_{10} values higher than 10 or lower than 1/10 represent strong evidence for the alternative and null hypotheses, respectively. A BF_{10} higher than 3 and lower than 1/3 represents moderate evidence for the alternative and null hypotheses, respectively. A BF_{10} between 3 and 1/3 reflects weak or anecdotal evidence for either hypothesis (Keysers, Gazzola, & Wagenmakers, 2020).

2.7.3 Correlation analysis

To test for a linear association between behavioral and neural sentential contextual facilitation, repeated-measures correlation was assessed using the "rmcorr" R package (Bakdash & Marusich, 2017). Repeated-measures correlation fits a linear model between two variables while controlling for non-independence within participants. In the present study, it was used to estimate the common intra-individual regression slope between SCF of RTs and SCF of 42-Hz ASSR, while allowing the intercepts to vary across participants.

3 Results

As expected after the task training, participants were able to perform both tasks successfully. On average, they correctly detected between 94% and 86% of the targets. Higher accuracies were observed in the speech task (on average 93%) than the distractor task (on average 87%). To assess neural tracking of hierarchical linguistic structures, we analyzed neural responses at the sentence rate (0.69 Hz), its harmonics (1.39 Hz and 2.08 Hz) and word rate (2.78 Hz). As shown in Fig. 2A, participants

reliably showed spectral peaks at these rates (compared to the average spectral amplitude of the six neighboring frequency bins: $p < .05$, paired t-test, FDR corrected), indicating participants could successfully track the sentence and word structures. In the unstructured speech condition, participants showed a spectral peak only at the word rate (Fig. 2B), and the ASSR at the sentence rate and its harmonics were significantly smaller than in structured speech condition (paired t-test, FDR corrected).

Fig. 3 shows participants' neural responses for each condition and each word position, averaged across channels showing the strongest sentence tracking. Prominent ASSRs at each tagging frequency (25 Hz and 42 Hz) were observed for all conditions and positions (spectral peaks at tagging frequency $>$ average spectral amplitude of the six neighboring frequency bins: $p < .05$; paired t-test, FDR corrected). These results indicate that auditory processing of the speech and distractor stimuli could be reliably assessed and separated. Analysis of the 42-Hz ASSR at each individual electrode revealed that neural responses to individual words were most prominent at central-frontal scalp regions, consistently across conditions and word positions (Fig. 4). Thus, the central-frontal channels that showed the strongest sentence tracking and were selected for further analysis (Fig. 2A) also showed strong 42-Hz ASSRs.

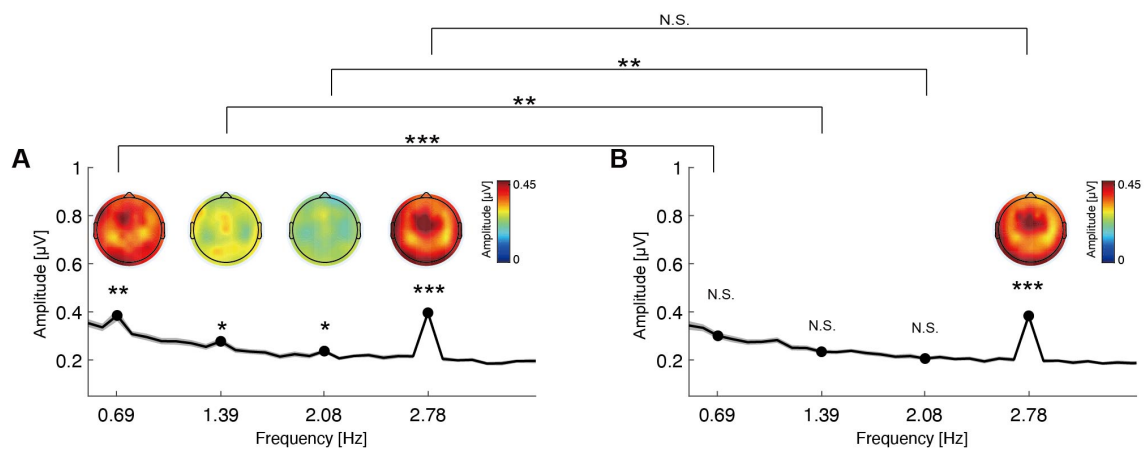


Figure 2. Neural responses to hierarchical linguistic structures. (A). The plot shows spectral responses averaged across all EEG channels to structured speech in the speech task as a function of frequency. Asterisks indicate significant responses at the sentence rate (0.69 Hz), word rate (2.78 Hz), and the second and third harmonics (1.39 Hz, 2.08 Hz) of the sentence rate relative to the average response at the six neighboring frequency bins, reflecting participants' detection of the sentence and word structures. The strongest sentence tracking was observed in a central-frontal scalp area. (B). Same as panel A, but for unstructured speech in the speech task. A significant response was observed only at the word rate (2.78 Hz), reflecting tracking of the word structure. Responses at the sentence rate and its harmonics were non-significant and significantly smaller than in structured speech (panel A). Black lines and shaded areas represent mean \pm sem across participants. N.S. non-significant, * $p < .05$, ** $p < .01$, *** $p < .001$, FDR corrected.

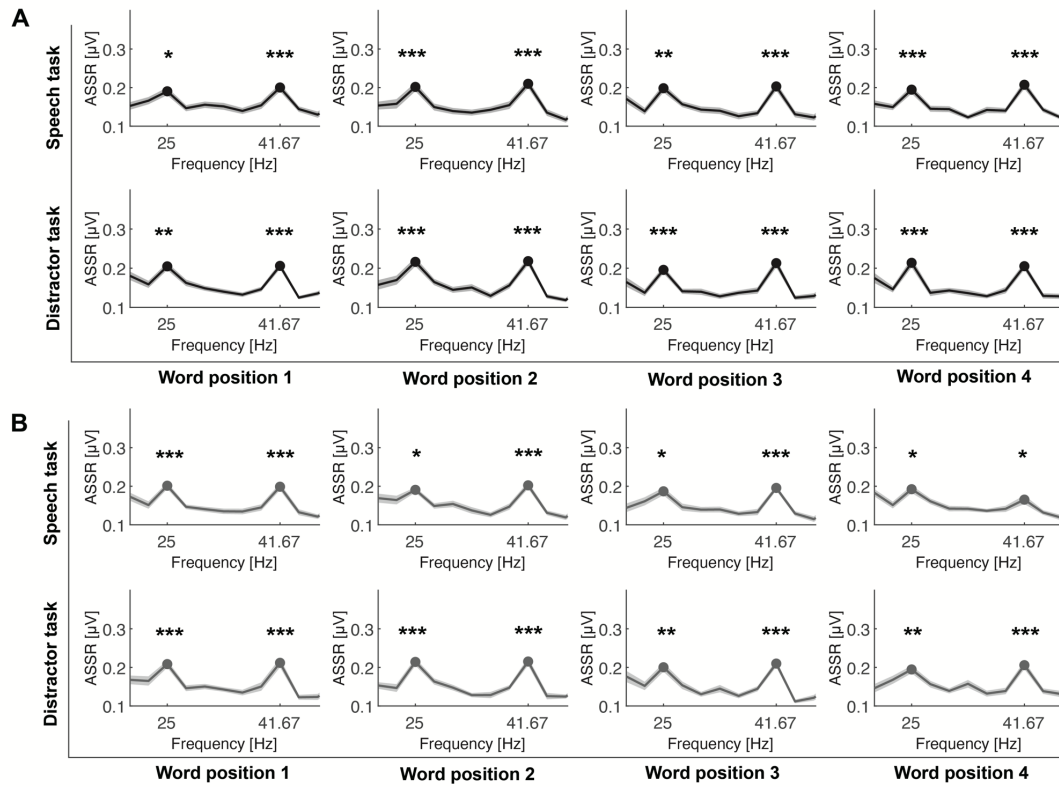


Figure 3. Neural responses to speech and distractor for each condition. Each plot shows ASSR in the range of the tagging frequencies (25 Hz and 42 Hz) averaged across channels in the central-frontal area. Asterisks indicate significant responses at the tagging frequency relative to the average responses at the six neighboring frequency bins. (A). Rows represent the two main experimental conditions involving structured speech stimuli (1st: speech task; 2nd: distractor task). Columns represent the four word positions. (B). Same as (A), but for the acoustic control condition (shuffled, unstructured speech) after unshuffling responses. Black lines and shaded areas represent mean \pm sem across participants. * $p < .05$, ** $p < .01$, *** $p < .001$.

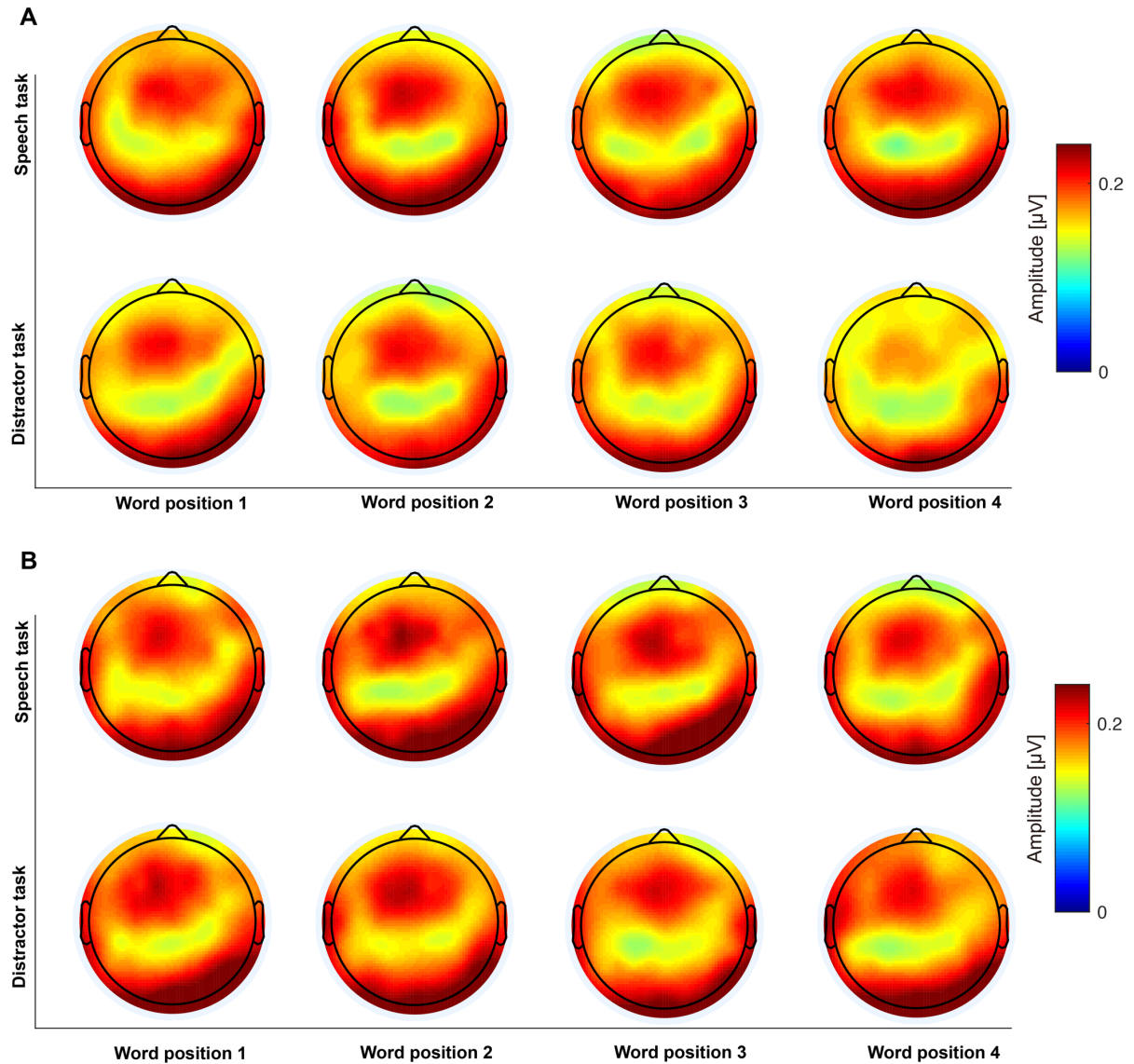


Figure 4. Topographic maps of neural responses to speech for each condition. Each plot shows the spatial distribution of the 42-Hz ASSR across the scalp. (A). Rows represent the two main experimental conditions involving structured speech stimuli (1st: speech task; 2nd: distractor task). Columns represent the four word positions. (B). Same as (A), but for the acoustic control condition (shuffled, unstructured speech) after unshuffling the responses. The 42-Hz ASSR was most prominent in frontocentral regions.

3.1 Buildup of sentential contextual facilitation of auditory word processing during sentence tracking

To assess the word-position effect during sentence tracking at the linguistic level, we first compared behavioral responses to target words presented at the different word positions. We found that SCF (a measure of sentential context effects controlled for word phonetics, frequency, and familiarity, see *Methods*) increased monotonically across word positions, reflecting a gradual decrease of RTs toward the end of sentences (Fig. 5A). This observation of an across-word pattern in sentential contextual facilitation was statistically confirmed by a one-way ANOVA including word position as a four-level

factor, which revealed a main effect of word position on SCF ($F_{3,60} = 30.86$, $p < .001$, $\eta^2_p = 0.61$). In line with this, a corresponding Bayesian ANOVA revealed a BF_{10} of 5.03×10^{10} , thus providing decisive evidence in favor of the main effect model compared to the null model (i.e., no word-position effect). This word-position effect shows that sentential context gradually accelerated the perceptual processing of words at later positions in the sentences.

Applying the same analysis (one-way repeated measures ANOVA, Bayesian ANOVA) to the neural responses to individual words revealed a similar across-word pattern. More specifically, SCF of the 42-Hz ASSR monotonically increased across word positions (Fig. 5B). This observation of an across-word pattern in sentential contextual facilitation was supported by strong evidence for a main effect of word position on SCF ($F_{3,60} = 5.23$, $p = .003$, $\eta^2_p = 0.21$, $BF_{10} = 22.17$). These results show that sentential context gradually modulated the cortical word-processing in the sentences. Interestingly, the observed effect is positive (i.e., larger responses at later word positions), which we further address in the Discussion.

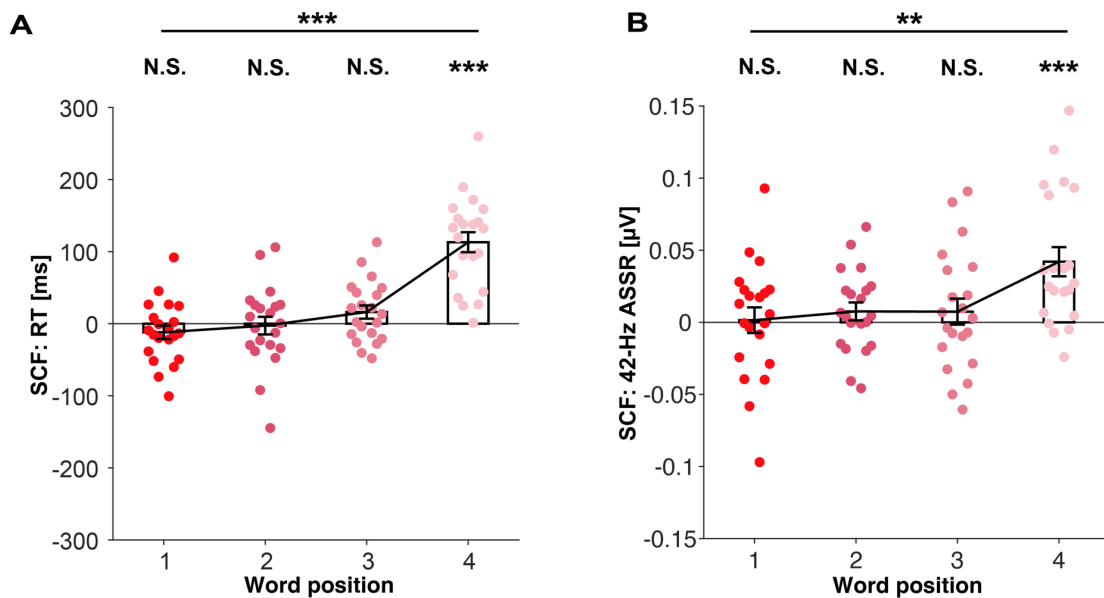


Figure 5. Across-word pattern of sentential contextual facilitation: behavioral and neural responses to individual words. (A). The bar plots show sentential contextual facilitation of participants' reaction time to individual words (structured speech stimuli minus control stimuli) as a function of word position within attended sentences. SCF of RTs increased monotonically. Note that positive SCF values represent accelerated RTs and negative values represent decelerated RTs. Therefore, sentential context gradually accelerated behavioral responses, revealing a word-position effect on linguistic word processing. (B). Same as (A), but for neural responses to words. Sentential context resulted in stronger ASSR amplitudes at later word positions. The significance marker above each bar indicates whether SCF was significantly larger than zero (corrected for multiple comparisons). Significant sentential contextual facilitation was observed in the last word position for both behavioral and neural responses. Red dots represent individual participants. Bars and error bars represent mean \pm sem across participants. N.S. non-significant, ** $p < .01$, *** $p < .001$.

Statistical analysis of Sen's slope (a nonparametric estimate of slope; see Methods) revealed a significant positive difference from zero for behavioral SCF and neural SCF (RT: $\beta = 39.5 \pm 5.7$, mean \pm sem; paired t-test: $t_{20} = 6.89$, $p < .001$; Bayesian t-test: $BF_{+0} = 3.40 \times 10^4$; ASSR: $\beta = 0.009 \pm 0.0031$, mean \pm sem; paired t-test: $t_{20} = 3.00$, $p = .005$; Bayesian t-test: $BF_{+0} = 13.54$), indicating significant upward trends across word positions. These results support our conclusion that SCF increased monotonously across word positions.

To further test the effects of sentential contextual facilitation on each word position, we compared SCF at each position to zero. We found that both behavioral SCF and neural SCF were significantly larger than zero at the last word position (RT: $t_{20} = 8.17$, $p < .001$, FDR corrected, $BF_{+0} = 3.48 \times 10^5$; ASSR: $t_{20} = 4.15$, $p < .001$, FDR corrected; $BF_{+0} = 135.00$), revealing that context facilitates both behavioral and neural responses to words especially at the end of the sentence.

3.2 Potential effect of attention on the buildup of sentential contextual facilitation

To explore whether the observed buildup of sentential contextual facilitation during sentence tracking requires listeners to pay selective attention to the speech, we tested whether the neural word-position effect persisted even when participants withdrew their attention from the speech stimuli. Contrary to the results above, we found no evidence for the main effect of word-position on SCF of the 42-Hz ASSR when participants performed the distractor task (one-way repeated measures ANOVA: $F_{3,60} = 0.31$, $p = .82$, $\eta^2_p = 0.015$; Bayesian ANOVA: $BF_{10} = 0.094$, Fig. 6). A two-way ANOVA with factors word position and attention revealed a word position \times attention interaction effect on SCF, however, the evidence for the interaction effect is weak ($F_{3,60} = 3.07$, $p = .037$, $\eta^2_p = 0.13$, $BF_{incl} = 0.89$ [comparing the model that contains the effect to an equivalent model without the effect]). These results suggest that the buildup of sentential contextual facilitation during sentence tracking depends on the listener's attention to the speech.

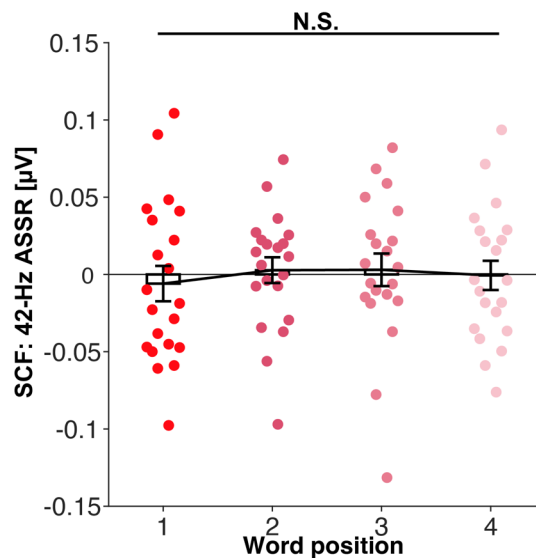


Figure 6. Across-word pattern of sentential contextual facilitation: neural responses to unattended individual words. The bar plots show sentential contextual facilitation of participants' neural responses to individual words (SCF: structured speech stimuli minus control stimuli) as a function of word position within unattended sentences. Contrary to the significant buildup observed during the speech task (Fig. 5B), no significant word-position effect on SCF was observed when participants

withdrew their attention from the speech to perform the distractor task. Red dots represent individual participants. Bars and error bars represent mean \pm sem across participants. N.S. non-significant.

3.3 No transfer of sentential contextual facilitation to auditory processing of concurrent sound streams

We further explored whether the observed buildup of sentential contextual facilitation during sentence tracking transfers to the processing of concurrent sound streams. To this end, we assessed whether the sentential context facilitated also participants' responses to the distractor tone. We found that SCF of RTs to the distractor tone was nearly zero at each word position and did not differ significantly across these positions (one-way repeated measures ANOVA: $F_{3,60} = 1.78$, $p = .16$, $\eta^2_p = 0.08$; Bayesian ANOVA: $BF_{10} = 0.42$, Fig. 7A). Moreover, the latter non-significant across-word variations were significantly smaller than those observed in responses to the speech (interaction word position \times sound stream: $F_{3,60} = 27.33$, $p < .001$, $\eta^2_p = 0.577$, $BF_{incl} = 9.59 \times 10^7$).

Consistent with this behavioral result, we found no main effect of word position on neural responses to the distractor tone, as measured by SCF of the 25-Hz ASSR (one-way repeated measures ANOVA: $F_{3,60} = 1.04$, $p = .38$, $\eta^2_p = 0.05$; Bayesian ANOVA: $BF_{10} = 0.88$, Fig. 7B). Similar to the behavioral results above, a two-way ANOVA revealed a significant word position \times sound stream interaction ($F_{3,60} = 2.97$, $p = .039$, $\eta^2_p = 0.129$, $BF_{incl} = 1.06$). In sum, these results indicate that the observed buildup of sentential contextual facilitation on auditory word processing did not transfer to the processing of the concurrent distractor tone.

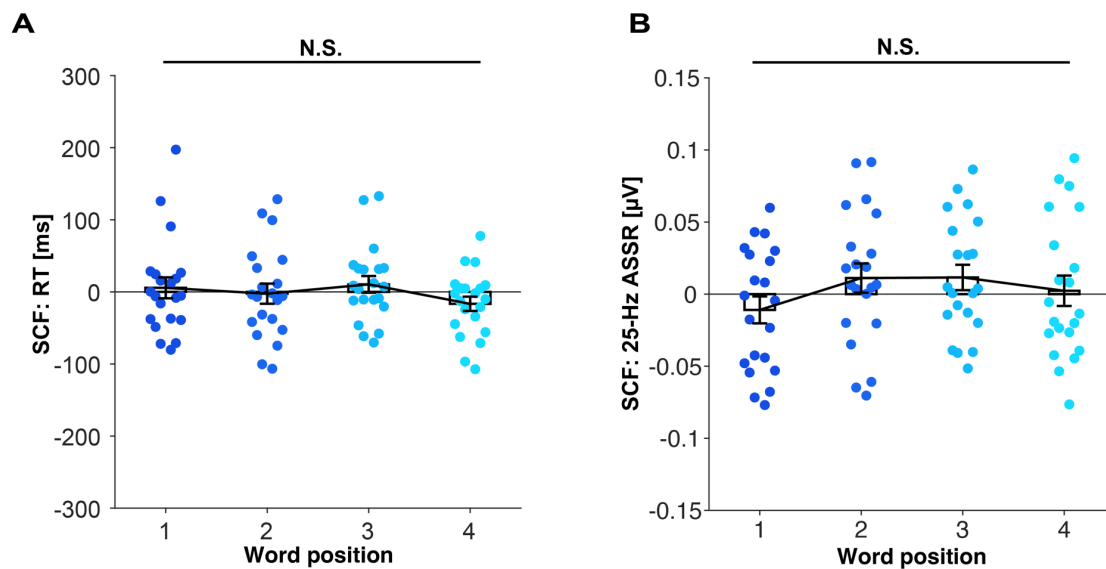


Figure 7. Across-word pattern of sentential contextual facilitation: behavioral and neural responses to the distractor tone. (A). The bar plots show sentential contextual facilitation of participants' reaction time to the distractor tone (SCF: structured speech stimuli minus control stimuli) as a function of word position within unattended sentences. No effect of word position on SCF was observed. (B). Same as (A), but for neural responses to the distractor tone (SCF of 25-Hz ASSR). Blue dots represent individual participants. Bars and error bars represent mean \pm sem across participants. N.S. non-significant.

3.4 Relation between sentential contextual facilitation of neural and behavioral responses to speech

To explore whether the sentential contextual facilitation of behavioral and neural responses across word positions were functionally coupled, we tested the correlation between SCF of RTs and SCF of 42-Hz ASSR (same data as in Fig. 5). A repeated-measures correlation analysis revealed a significant positive correlation between SCF of RTs and SCF of 42-Hz ASSR ($r_{rm} = 0.414$, $p < .001$, 95% CI: 0.187 to 0.599, Fig. 8). This neural-behavioral result indicates that the contextual facilitation of cortical processing of individual words was associated with a corresponding facilitation of the behavioral detection to these words, suggesting a link between acoustic and linguistic levels of auditory word processing.

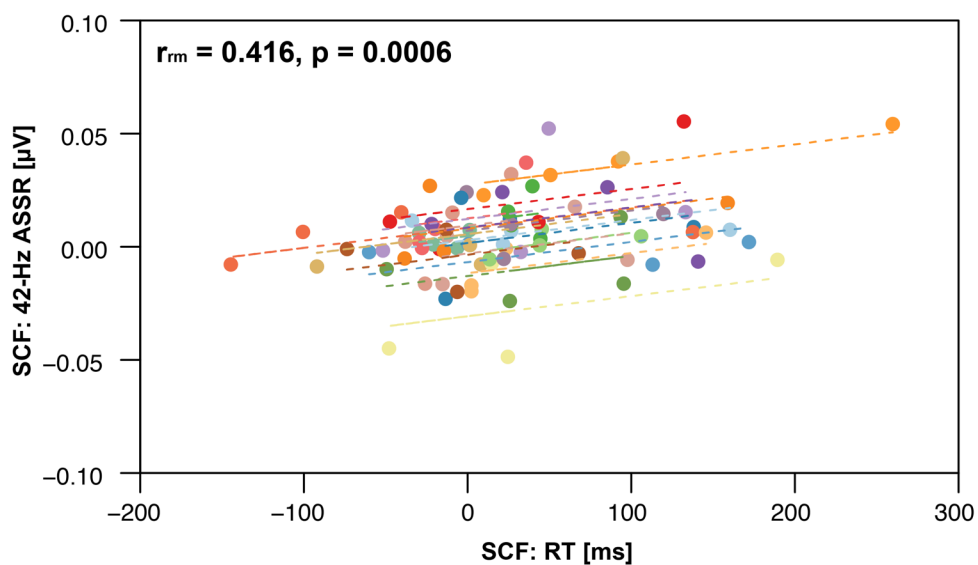


Figure 8. Relation between sentential contextual facilitation of behavioral and neural responses to speech. The scatterplot shows results from a repeated-measures correlation analysis testing for a functional coupling between SCF of RTs and SCF of 42-Hz ASSR to words across word positions. Dots with the same color represent responses to words at different positions from the same participant. The corresponding lines show the repeated-measures correlation fit for each participant. Correlation coefficient r_{rm} and p-value describe, respectively, the strength and statistical significance of the common intra-individual coupling.

3.5 Effect of syntax on the buildup of sentential contextual facilitation

In an additional exploratory analysis, we investigated whether the buildup of sentential contextual facilitation during sentence tracking depends on the syntax of the speech (i.e., syntax NVAN vs. AANV). A two-way ANOVA including word position (word interval: 1st, 2nd, 3rd, or 4th) and speech syntax (syntax NVAN and AANV) as factors revealed weak evidence for an interaction (word position \times syntax) effect on SCF of RTs ($F_{3,60} = 3.24$, $p = .028$, $\eta^2_p = 0.14$, $BF_{incl} = 1.61$, Fig. 9). Post-hoc comparisons for each syntax confirmed a main effect of word position on SCF in syntax NVAN as well as syntax AANV (syntax NVAN: $F_{3,60} = 25.07$, $p < .001$, $\eta^2_p = 0.56$, $BF_{10} = 3.67 \times 10^8$; syntax AANV: $F_{3,60} = 9.23$, $p < .001$, $\eta^2_p = 0.32$, $BF_{10} = 1.92 \times 10^3$). Statistical analysis of Sen's slope revealed an upward trend across word positions

for each syntax (syntax NVAN: $\beta = 52.0 \pm 7.8$; syntax AANV: $\beta = 29.7 \pm 6.6$; mean \pm sem) that was significantly stronger in syntax NVAN than syntax AANV (paired t-test, $t_{20} = 2.40$, $p = .026$). Post-hoc pairwise comparisons between the two syntaxes at each individual position showed no significant difference at any position ($p > .05$, FDR corrected). These results suggest that the observed word position \times syntax interaction effect on SCF of RTs reflects differences in across-word trend, rather than a difference at a single specific word position. Applying the same two-way ANOVA as above to the neural data revealed no such interaction effect on SCF of 42-Hz ASSR ($F_{3,60} = 1.26$, $p = 0.30$, $\eta^2_p = 0.059$, $BF_{incl} = 0.082$). In sum, these exploratory results suggest that the speech syntax may influence the temporal shape of sentential contextual facilitation of linguistic word processing.

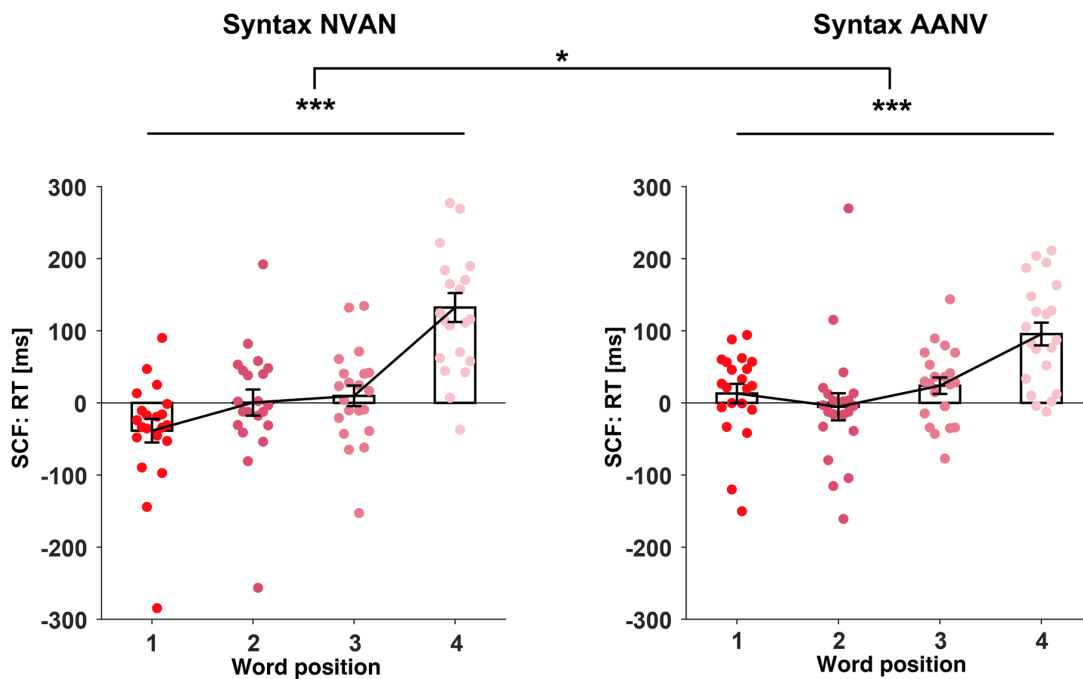


Figure 9. Across-word pattern of sentential contextual facilitation: behavioral responses to individual words in sentences of syntax NVAN and syntax AANV. The bar plots show sentential contextual facilitation of participants' reaction time to individual words (structured speech stimuli minus control stimuli) as a function of word position within attended sentences of syntax NVAN (left plot) and syntax AANV (right plot). Overall, sentential context gradually accelerated behavioral responses at later word positions, but the exact across-word pattern depended significantly on the syntax.

4 Discussion

In this study, we investigated the dynamics of auditory word processing during the tracking of continuous meaningful speech. We found that (i) sentential context alone incrementally facilitates behavioral and neural responses to individual words as the sentence unfolds. This sentential contextual facilitation (ii) depends on listeners' endogenous selective attention to the speech, and (iii) affects selectively the processing of the speech stream, not that of a concurrent sound stream, (iv) may be shaped by the syntax of the speech. Additionally, we found that the facilitation of behavioral and neural responses to individual words in our paradigm were coupled. Overall, these results provide evidence for the hypothesis that the dynamics in auditory processing during sentence tracking can be

driven exclusively by buildup of top-down factors, specifically sentential predictions derived from the speech.

4.1 *Sentential predictability elicits word-processing dynamics*

Our observation that sentential context incrementally facilitates perceptual word detection during sentence tracking aligns with previous work showing that word processing may be accelerated across word positions in sentences (Lam et al., 2016; Marslen-Wilson & Tyler, 1975; Van Petten & Kutas, 1990, 1991). Our finding of a word-position effect on word processing that originates exclusively from sentential context (i.e., in absence of variations in word phonetics, frequency, familiarity, and overt prosody) is novel. It provides strong evidence to the notion that the facilitation of the processing of later-occurring words can be attributed to a gradual increase in sentential predictability (Clark, 2013; DeLong, Urbach, & Kutas, 2005; Hagoort & Indefrey, 2013; Van Petten & Kutas, 1991; Van Petten & Luka, 2012). As a sentence unfolds, semantic context incrementally builds up, and the accruing context increases the predictability of later words in the sentence. Consequently, listeners in our study may have recognized the later words more quickly than the earlier words because their meaning was more predictable.

While syntactic predictions can influence linguistic analysis of individual words during sentence tracking (see Introduction), they unlikely caused the word-position effects observed in our study. We kept the syntax constant across all sentences within a given block, implying no variations in syntactic predictability across word positions. Alternatively, the observed facilitation at later words may have been mediated by covert prosody (prosodic boundaries internally projected onto the stimulus in the absence of overt prosodic phrase boundary markers) that listeners possibly generated from the sentential context (Breen, 2014; Glushko, Poeppel, & Steinhauer, 2020). Even though our study cannot distinguish between these two accounts (semantics vs. covert prosody), it does unequivocally demonstrate a purely top-down influence on word processing that originates from sentential context. Similar position effects have also been reported at the level of syllables. EEG studies found that word-initial syllables elicit a larger N1 component than word-medial syllables and the processing of the syllables is modulated by predictability (Astheimer & Sanders, 2011; Sanders & Neville, 2003). Thus, studies at syllable and word levels jointly demonstrate the temporal dynamics of speech processing and support a role of predictability in these dynamics.

Consistent with our behavioral results, the analysis of neural responses to speech revealed a significant word-position effect. We found that sentential context incrementally facilitates cortical processing of auditory words (as measured with ASSR) as the sentence unfolds. As explained above, the primary origin of these dynamics in cortical word processing may be attributed to semantic predictability. This interpretation is in line with neural findings showing effects of semantic comprehension on much slower phase-locked cortical responses to natural speech envelope (Ahissar et al., 2001; Kaufeld et al., 2020; Park, Ince, Schyns, Thut, & Gross, 2015; Peelle, Gross, & Davis, 2013; Pefkou, Arnal, Fontolan, & Giraud, 2017). Our measure of cortical word processing (42-Hz ASSR) likely reflects phase-locked responses to the artificially-induced rapid modulations of the word waveform (Kuwada, Batra, & Maher, 1986; Picton, John, Purcell, & Plourde, 2003; Rodriguez, Picton, Linden, Hamel, & Laframboise, 1986). Therefore, the neural results may indicate a primary effect of sentential predictability on cortical processing of lower-level acoustic speech-signal features.

The positive direction of the effect (i.e., higher semantic predictability leading to stronger auditory-evoked response) may at first sight seem to conflict with predictive coding accounts, that typically demonstrate reduced neural activity for highly predictable content (Grisoni, Tomasello, & Pulvermüller, 2021; Kutas & Federmeier, 2011). However, we should emphasize that in our finding predictability led to increased ASSR, representing the processing of the amplitude modulations of the acoustic signal, rather than linguistic processing. This suggests that increased predictability of linguistic content allowed listeners to shift processing resources from the linguistic analysis of the ongoing

speech signal to its auditory analysis, which probably led to a more faithful auditory cortical representation of the speech signal and accelerated word recognition.

We further observed a significant correlation between the facilitation in behavioral and neural responses to individual words: words that were detected more quickly tended to elicit stronger word processing. This suggests that the enhancement of neural responses to the acoustic speech signal improved the perceptual detectability of the target word.

4.2 *Potential effect of selective attention on the buildup of sentential contextual facilitation*

The sentential contextual facilitation of neural word processing was observable only when listeners paid selective attention to the speech. This extends previous findings showing contributions of selective attention to the processing of individual words. For example, speech studies using continuous isochronous syllable sequences have shown that listeners' attention may be required for the grouping of consecutive syllables into words (Ding et al., 2018; Makov et al., 2017). Moreover, it has been shown that listeners detect target words in sentences more rapidly when they are semantically primed to focus their attention on these words (Cutler & Fodor, 1979). Our results extend these findings to the grouping of words into sentences and, more importantly, the incremental sentential contextual facilitation of auditory word processing. As explained above, the observed word-processing dynamics likely originated from sentential predictability, not from phonetic or overt prosodic effects that may be more immune to selective attention (Bosker, Sjerps, & Reinisch, 2020). As such, it is likely that our listeners grouped the words into sentences and could thereby extract contextual information only when they paid attention to the speech. Whether selective attention is generally necessary for sentential predictions—and, by extension, word-processing dynamics—remains unclear from our study and would require testing a wider range of speech stimuli and tasks.

4.3 *Sentential contextual facilitation does not transfer to processing of concurrent sound streams*

We observed no sentential contextual facilitation of the processing of the concurrent distractor tone (or significant temporal changes therein), suggesting that the dynamics in word processing did not transfer to the processing of the distractor. In contrast, some previous studies observed a word-position effect on acoustic event detection during speech processing; for example, shorter RTs were found for clicks occurring during later vs. early words (Holmes & Forster, 1970; Lobina et al., 2018). The difference to these previous results may reflect methodological differences: in the previous studies, the concurrent sound was an occasional brief acoustic event and participants were required to pay attention simultaneously to a click and the meaning of the ongoing speech. In contrast, our study used a continuous distractor tone encouraging auditory stream segregation and two separate tasks requiring no division of attention. Thus, our null result showing no effect of sentential speech context on the processing of a simultaneous distractor tone may indicate that sentential predictions affect only the processing of the sound stream from which these predictions are derived, not input that is perceptually separate from that stream. Put differently, sentential contextual facilitation of acoustic word processing (i.e., processing of the amplitude variations of the sound wave of words) probably occurs at a later stage than the perceptual segregation of the speech from concurrent streams, which may occur within ~200ms after speech onset (Alain, Arsenault, Garami, Bidelman, & Snyder, 2017; Bidelman & Alain, 2015; Bidelman & Yellamsetty, 2017) in auditory structures as early as the cochlear nucleus (Pressnitzer, Sayles, Micheyl, & Winter, 2008). It should be noted that, in contrast to the speech stream, the ongoing tone contained no identifiable structure, implying that listeners could not extract valid predictions from it. A temporally structured tone might have made it more likely to observe a significant temporal (across-word) pattern also in tone processing.

4.4 *Speech syntax modulates the buildup of sentential contextual facilitation*

Our exploratory analysis of syntax effects revealed that the buildup of sentential contextual facilitation may be influenced by the speech syntax. Syntax modulated the temporal shape of the behavioral, but not the neural, word-position effect, which may be related to asymmetries between our behavioral and neural measures. The behavioral measure probably captured linguistic processes, whereas the neural measure captured more acoustics-related processes. Therefore, the syntax probably affected the facilitation of linguistic, not acoustic, word processing.

A potential explanation for this side observation may be differences in the hierarchical syntactic structure of NVAN and AANV. NVAN has a more specific verb phrase (consisting of a verb, adjective, and noun, e.g. “eat fresh fish”), whereas AANV has a more specific noun phrase (consisting of an adjective, adjective, and noun, e.g. “Two big dogs”). Listeners probably built an internal representation of the hierarchical syntactic structure during speech processing (Friederici, 1995, 2002), and the differences in these structures might have resulted in differences in the buildup of this representation or its utilization for the analysis of the incoming speech signal. However, this interpretation is tentative and requires thorough verification with a better-suited study design using a larger number of syntactic structures and combining sentences of various lengths.

4.5 *Conclusion*

In summary, our study reveals that the acoustic and linguistic processing of auditory words during sentence tracking builds up dynamically and these dynamics can be driven exclusively by top-down factors, in particular sentential predictions derived from the processed speech. These factors may depend on the listener’s selective attention to the speech and affect only the processing of speech, not that of perceptually separate sound streams. It appears that auditory and semantic processes during sentence tracking interact reciprocally: auditory processing of individual words may inform the formation of syntactic and semantic structure, and predictions derived from these structures may modulate the auditory processing of the words, provided that the listener pays selective attention to the speech stream. These conclusions could be drawn due to our control of phonetics and prosody, at the expense of the naturalness of our auditory stimuli. Future work is encouraged to explore whether these findings generalize to natural speech.

Data availability

Data and analysis scripts have been deposited in Open Science Framework (<https://osf.io/qzvsn/>).

Author contributions

Min Wu: Conceptualization, Investigation, Formal analysis, Data curation, Methodology, Software, Funding acquisition, Writing – original draft. **Hans Rutger Bosker:** Conceptualization, Methodology, Writing – review & editing. **Lars Riecke:** Conceptualization, Methodology, Resources, Data curation, Supervision, Writing – original draft, Writing – review & editing.

Acknowledgements

This work was supported by Maastricht University and China Scholarship Council (CSC 201906320078 to M.W.).

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5 Supplementary materials

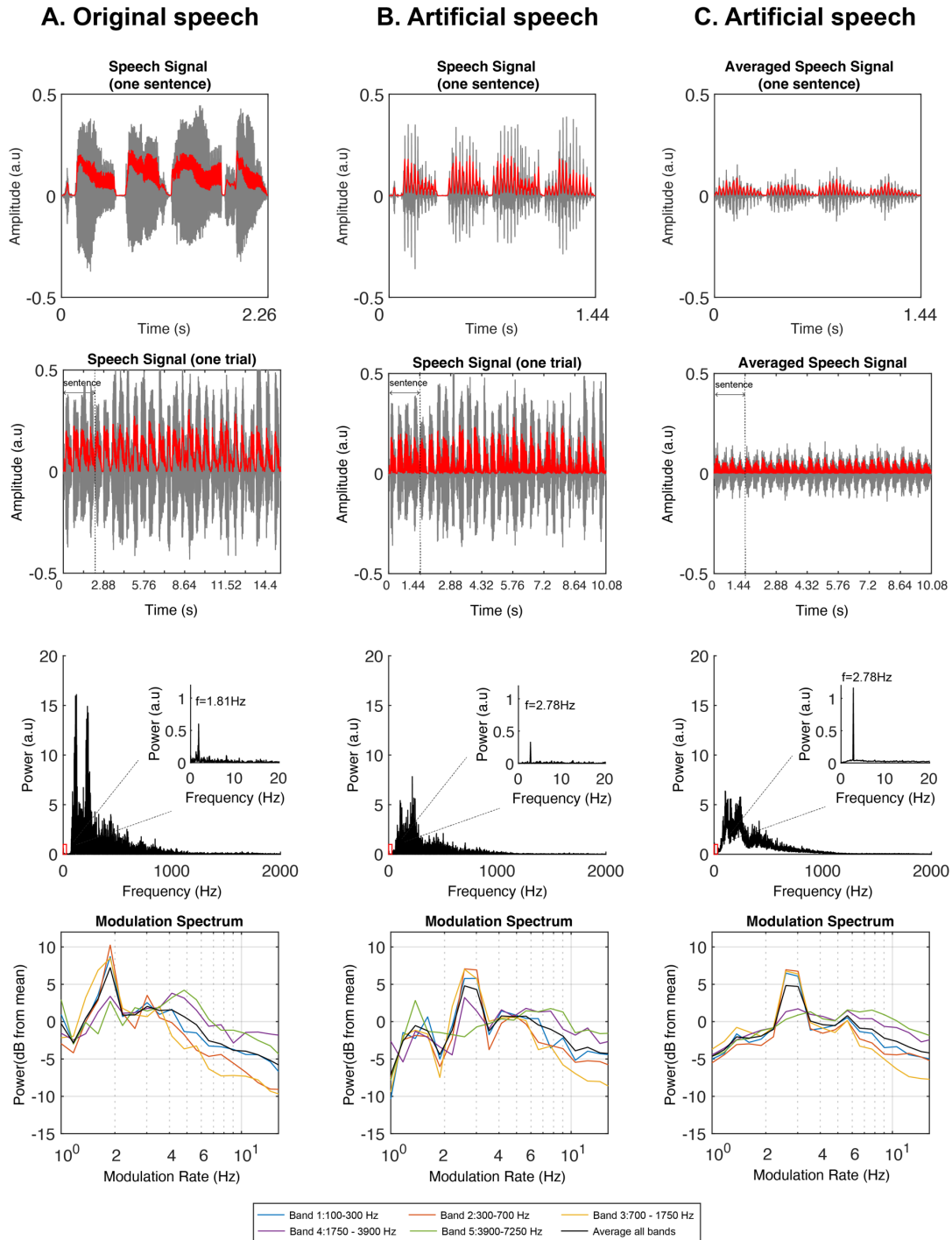


Figure S1. Speech acoustics. Panel A, B, and C show respectively an exemplary original excerpt, a time-compressed version of the same excerpt, and the average of all time-compressed excerpts. The first to fourth row show respectively the sound waveform of a single sentence (in gray, amplitude envelope is shown in red), the sound waveform of a single trial (seven sentences), the average power spectrum of all sentences, and the average power spectrum of the amplitude envelopes of all sentences (modulation spectrum, shown for the broadband envelope as well as for non-overlapping narrow envelope bands).

Chapter 3

Multimodal acoustic-electric trigeminal nerve stimulation modulates conscious perception

Based on

Wu, M., Auksztulewicz, R., Riecke, L. Multimodal acoustic-electric trigeminal nerve stimulation modulates conscious perception. Submitted.

Abstract

Multimodal stimulation has the potential to reverse pathological neural activity and alleviate symptoms in neuropsychiatric diseases. However, the reliability of this approach and the mechanisms through which it improves consciousness remain largely unknown. We investigated the effects of multimodal stimulation combining music stimulation with electrical trigeminal nerve stimulation in healthy human participants. We assessed conscious perception before and after acoustic-electric stimulation and investigated the mechanisms underlying the putative stimulation effects. Our results show that (1) acoustic-electric stimulation improves conscious tactile perception in healthy human participants without a concomitant change in auditory perception, (2) this improvement is caused by the interplay of the acoustic and electric stimulation rather than any of the unimodal stimulation alone, and (3) the effect of acoustic-electric stimulation on conscious perception correlates with inter-regional connection changes in a recurrent neural processing model. These findings provide evidence that multimodal acoustic-electric stimulation can promote conscious perception and offer insights into its underlying mechanisms.

Keywords

Multimodal stimulation, trigeminal-nerve stimulation, conscious perception, dynamic-causal modeling

1 Introduction

Consciousness is an important and ubiquitous aspect of our daily lives. Many pathologic brain states, such as epileptic seizures, stroke and encephalitis, can lead to impaired consciousness. In particular during the COVID-19 pandemic, up to 19.6% of patients with COVID-19 suffered from impaired consciousness (Mao et al., 2020; Romero-Sánchez et al., 2020). Consciousness impairment can have devastating consequences for safety and quality of life; therefore, it is particularly important to develop effective approaches to promote consciousness.

Over the past decades, an increasing variety of consciousness-promoting therapies have been proposed. These therapies include pharmacological treatments (e.g., zolpidem, apomorphine) (Sanz et al., 2019; Thonnard et al., 2013) and nonpharmacologic interventions, such as sensory or electric stimulation (Cooper, Scherder, & Cooper, 2005; Thibaut, Schiff, Giacino, Laureys, & Gosseries, 2019). A recent approach, inspired by the increasing knowledge of the neural mechanisms underlying consciousness, involves non-invasive electric stimulation of the trigeminal nerve (Fan et al., 2019; Wu et al., 2022). This cranial nerve projects facial sensation to the brainstem and the cortex, and connects to the reticular activating system, thalamus, insula, and somatosensory brain structures (Simpson et al., 1997)—regions which have been proposed to play a role in consciousness (Gallace & Spence, 2010; Schiff et al., 2007; Steriade, 1996).

Trigeminal nerve stimulation (TNS) has already been widely used in many neurological disorders (DeGiorgio et al., 2013; McGough et al., 2019) and first applications of it in consciousness research have yielded promising results. It has been observed that TNS of rats with impaired consciousness can upregulate neuropeptide hypocretin in the lateral hypothalamus and this activation can promote consciousness recovery (Zheng et al., 2021). In other animal models with traumatic brain injury, TNS has been demonstrated to enhance cerebral blood flow, protect the blood-brain barrier, and reduce brain edema (Chiluwal et al., 2017; Yang et al., 2022). Similar effects of TNS have been shown in human patients diagnosed with disorders of consciousness (DOC). In a single-case study, Fan et al. reported an improvement of consciousness after TNS in a DOC patient (Fan et al., 2019). Similarly, Dong et al. reported consciousness benefits after four weeks of TNS in eight out of 21 DOC patients (Dong, Tang, Fang, & Feng, 2022). Therefore, both animal and human findings suggest that TNS may be an effective approach for promoting the recovery of consciousness.

Compared to unimodal stimulation, multimodal stimulation has been shown to modulate more widespread brain regions and cause stronger neural activation within the multisensory regions (Godenzini et al., 2021; Markovitz, Smith, Gloeckner, & Lim, 2015; Marks et al., 2018). For example, combined acoustic and visual stimulation in Alzheimer's disease (AD) mice has been found to reduce amyloid load (a pathological hallmark of AD) across much broader cortical regions than acoustic or visual stimulation alone (Martorell et al., 2019). Similar benefits of multimodal stimulation have been observed in tinnitus studies, which found that combined acoustic and electric stimulation, but not unimodal stimulation, can reverse tinnitus-related pathological neural activity and alleviate tinnitus symptoms (Engineer et al., 2011; Marks et al., 2018). These findings suggest that multimodal stimulation allows for a more effective treatment of some cognitive disorders than unimodal stimulation.

Inspired by these findings, we recently conducted a stimulation study in which we combined rhythmic acoustic music stimulation and rhythmic transcutaneous electrical TNS in DOC patients (Wu et al., 2022). We found that multimodal acoustic-electric stimulation in the gamma band (40Hz) can promote both gamma neural activity and re-emergence of consciousness. Given the evidence above, multimodal acoustic-electric stimulation appears to be a promising approach for promoting consciousness. As the approach is still novel, its reliability and functional principle are still poorly understood and require further investigation.

In the aforementioned DOC patient studies, the level of consciousness was assessed by clinicians with a questionnaire, the Coma-Recover Scale Revised (CRS-R). Although this scale currently

constitutes the gold standard for consciousness assessment (Seel et al., 2010), it highly depends on the assessor's experience and can lead to a high rate of misdiagnosis in patients with motor impairment (Schnakers et al., 2009). An alternative measure that is commonly used in studies of neural correlates of consciousness exploits participants' subjective experience of sensory input, as quantified with perceptual performance on a near-threshold target-detection task (Eklund & Wiens, 2019). In this assessment, healthy participants are asked to report whether they are aware or unaware of a sensory stimulus that is repeatedly presented at a fixed intensity near the perceptual detection threshold. While this measure can serve merely as a proxy for consciousness, it has the advantage that it directly reflects the participant's subjective experience and can be readily coupled with objective measures. For example, neural markers of conscious perception can be identified by comparing neural responses to detected vs. undetected identical stimuli while controlling for confounding variations in sensory input. Results of previous EEG studies using this approach suggest that conscious perception involves two prominent event-related potential (ERP) components: an early negative component in sensory regions 120-200 ms after the stimulus onset and a late positive component in occipital-parietal regions 250-500 ms after the stimulus onset (Dembski, Koch, & Pitts, 2021; Eklund & Wiens, 2019; Mika Koivisto & Revonsuo, 2010). If multimodal acoustic-electric stimulation can reliably promote consciousness, one would expect it to effectively enhance these alternative measures of consciousness.

Despite the converging results of the aforementioned AD and tinnitus studies, the empirical evidence for stronger benefits from multimodal (compared with unimodal) stimulation for consciousness is still very limited. So far, consciousness benefits from multimodal stimulation have been investigated primarily by stimulating in different sensory modalities consecutively (Cheng et al., 2018; Megha, Harpreet, & Nayeem, 2013), rather than simultaneously (Wu et al., 2022). Thus, it is still unclear whether the consciousness benefit from multimodal acoustic-electric stimulation results from the multimodal nature of the stimulation. More generally, the mechanism by which the multimodal stimulation may improve consciousness is still unclear. Therefore, it remains to be determined whether and how consciousness benefits from multimodal acoustic-electric stimulation are driven by the acoustic or electric stimulation, or their simultaneous combination.

The present study aimed to investigate (1) whether the effect of multimodal stimulation combining music stimulation and electrical TNS (hereafter shortly called "acoustic-electric stimulation") on consciousness is reproducible in healthy participants using behavioral and neural measures of conscious perception in a target-detection task; (2) whether the effect is primarily driven by the combination of the acoustic and electric stimulation or any of the unimodal inputs alone; and (3) potential neural mechanisms underlying the putative effects of acoustic-electric stimulation. To achieve aims 1 and 2, we performed two experiments in healthy human participants using a double-blinded, randomized, crossover design. We applied acoustic-electric and acoustic-only stimulation (Experiment 1, Fig.1A), and electric-only and electric-sham stimulation (Experiment 2, Fig.1B). We assessed conscious perception before and after the stimulation based on participants' perceptual performance on tactile and auditory target-detection tasks and electroencephalography (EEG) responses to undetected vs. detected targets (Fig.2). To achieve aim 3, we applied a dynamic-causal modeling (DCM) approach to fit a biologically plausible neural network model to the EEG data. DCM is a useful tool to study the neural architecture underlying observed electrophysiological features in terms of effective connectivity (Stephan et al., 2010). Previous EEG studies have utilized this approach to investigate neural mechanisms of tactile conscious perception and found a potential role of recurrent neural processing in the cortex (Auksztulewicz & Blankenburg, 2013; Auksztulewicz, Spitzer, & Blankenburg, 2012). Previous consciousness studies have demonstrated that the disruption of connectivity is a candidate mechanism of impaired consciousness, and restoration of brain connectivity is accompanied by recovery of consciousness (Boly et al., 2011; Laureys et al., 2000). On this basis, we hypothesized that changes in effectivity connectivity might be the mechanisms by which acoustic-electric stimulation induces consciousness benefits. We analyzed the connectivity

parameters of the best-fitting neural network model to test whether acoustic-electric stimulation can modulate estimates of inter-regional cortical connections.

We hypothesized that (1) acoustic-electric stimulation elicits an improvement of conscious perception in healthy human participants, as indicated by increases in both detection performance and awareness-related ERP components after vs before the stimulation, (2) this improvement in conscious perception is larger after acoustic-electric stimulation than after acoustic or electric stimulation alone, and (3) the putative effect of acoustic-electric stimulation on consciousness correlates with inter-regional connection changes in a recurrent neural processing model.

2 Methods

2.1 Participants

Participants were recruited from the student population of Maastricht University. Fifty-four participants (42 females, 12 males; ages: 18-30 years) completed the study. Two participants with poor EEG data quality (Experiment 1, see section *Electrophysiology* for details) were excluded. The remaining 52 participants were included for further analysis: 26 participants in Experiment 1 and another 26 participants in Experiment 2. All participants reported normal hearing and no history of neurological or psychiatric disorders. No participants had contraindications to transcutaneous electric stimulation. Written informed consent was obtained prior to the experiment. Participants were compensated with study credits or monetary reward for their participation. The experimental procedure was approved by the local research ethics committee (Ethical Review Committee Psychology and Neuroscience, Maastricht University).

2.2 Study overview

The study included two double-blinded, randomized, crossover experiments. Each experiment consisted of two sessions separated by at least 48 hours. Each session comprised three phases: an assessment of conscious perception before stimulation (i.e., pretest), application of the stimulation, and an assessment of conscious perception after stimulation (i.e., posttest) (Fig.1). The only difference between two sessions was the type of applied stimulation: in Experiment 1, it was either acoustic-electric or acoustic-only, and in Experiment 2, it was either electric-only or electric-sham. The order of stimulation type was counterbalanced across participants. Participants and data collectors were blinded to stimulation type throughout the experiment. During the pretest and posttest phases, behavioural responses (Experiments 1 and 2) and neural responses (Experiment 1) were measured in tactile (Experiments 1 and 2) and auditory (Experiment 1) detection tasks.

2.3 Experiment 1

2.3.1 Conscious perception assessment

To allow assessing conscious perception during the pretest and posttest phases, a target-detection task adopted from previous studies was used (Auksztulewicz & Blankenburg, 2013; Eklund & Wiens, 2019; Sanchez, Hartmann, Fusca, Demarchi, & Weisz, 2020; Schroder et al., 2021). Tactile stimuli were 1-ms biphasic square wave pulses generated by a constant current stimulator (DS5, Digitimer). The tactile stimuli were delivered via Ag/AgCl electrodes adhered to the tip of the left index finger to stimulate the median nerve. Auditory stimuli were 1000-Hz pure tones with a duration of 100 ms (including 5 ms fade-in and 5 ms fade-out), embedded in continuous white noise (44.8 dB SPL). The auditory stimuli were presented binaurally via insert earphones.

Prior to the main assessment, individual tactile and auditory target-detection thresholds were measured with a two-step procedure involving the method of adjustment (step 1) and the method of constant stimuli (step 2). In step 1, participants were asked to increase the current intensity from 0.3 mA (in steps of 0.1 mA) to the lowest stimulus intensity at which they could detect the electric pulse. In step 2, participants were exposed to pulses with various intensities (ten equidistant levels centred on the current intensity determined in the first step, with an increment size 0.06 mA). A total of 100 trials (10 for each intensity) were presented in random order. Participants responded with a button response on each trial to indicate whether they had detected the pulse or not. The obtained data were fitted with a logistic psychometric function, from which three intensities yielding the following performance levels were derived: 1% detection rate (intensity 1), 50% detection rate (intensity 5, equal to detection threshold), and 99% detection rate (intensity 9). Intensities 1 and 9 were used to define the intensity range that was presented in the subsequent assessment of conscious perception. The auditory-threshold assessment followed the same procedure as the tactile-threshold assessment above, with the exceptions that the loudness of the tone started at an audible level (45 dB) and decreased in steps of 4 dB in the first step, and the increment was 2 dB in the second step.

After the threshold measurements, four blocks of detection-task trials were presented while EEG was recorded. Each block contained 150 trials and lasted ~9 min. Two blocks contained only tactile trials and two blocks contained only auditory trials. The presentation order was TATA or ATAT (T: tactile; A: auditory) and counterbalanced across participants. In each block, the 150 trials were presented at nine intensities, which were equidistantly spaced between 1% (intensity 1) and 99% (intensity 9) detection rate, as described above. The number of trials presented at each intensity level followed a normal distribution to maximize the trials with intensities near the detection threshold (Fig.2B).

Each block started with the presentation of a task instruction on the screen, which instructed participants to perform the tactile or auditory task. Each trial started with a white central fixation cross on a black screen for 2000 ms. A tactile or auditory stimulus was applied with a random delay between 1000-1500 ms after the fixation onset. Next, a response screen was displayed, instructing participants to report whether they had detected a stimulus or not by pressing one of two buttons ("1" or "2") within 1500 ms. No feedback on task performance was given. Buttons "1" and "2" in first two blocks represented "Yes: detected" and "No: undetected". To control for motor-response mapping, the mapping was reversed in blocks 3 and 4 with "1" and "2" indicating "No: undetected" and "Yes: detected" respectively (Fig.2A). Participants could take a break after each block for as long as they needed.

Two brief practice blocks of 30 trials were presented prior to the main assessment to familiarize participants with the stimuli and tasks. The threshold measurement and practice procedures were conducted only in the pretest phase of each session.

2.3.2 Acoustic and electric stimulation

Acoustic stimulation consisted of eight pieces of Japanese music. To control for a potential learning effect, the eight pieces of music were divided into two sets that were played in individually randomized order in the two sessions. 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 20-min stream of music. To enforce rhythmic brain activity at gamma frequency, the sequence was amplitude-modulated at a frequency of 40 Hz (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 comfortable sound level (70.8 dB SPL).

Electric stimulation consisted of the non-invasive application of alternating currents to the participant's face to facilitate rhythmic trigeminal nerve activity. Analogously to the acoustic stimulation, the current waveform was a sinusoid with a frequency of 40 Hz. 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 participant's face to stimulate the second and third branches of the trigeminal nerve (i.e., the maxillary nerve and the mandibular nerve, respectively). Based on previous research and pilot tests (McGough et al., 2019), the intensity of the current was fixed to ± 4 mA. The onset/offset of the current was ramped up/down using 5-s long ramps. The electrodes were adhered to the participant's skin using conductive paste and the impedance was kept below 10 k Ω . The electric stimulation was delivered using battery-powered DC stimulators (NeuroConn, Germany).

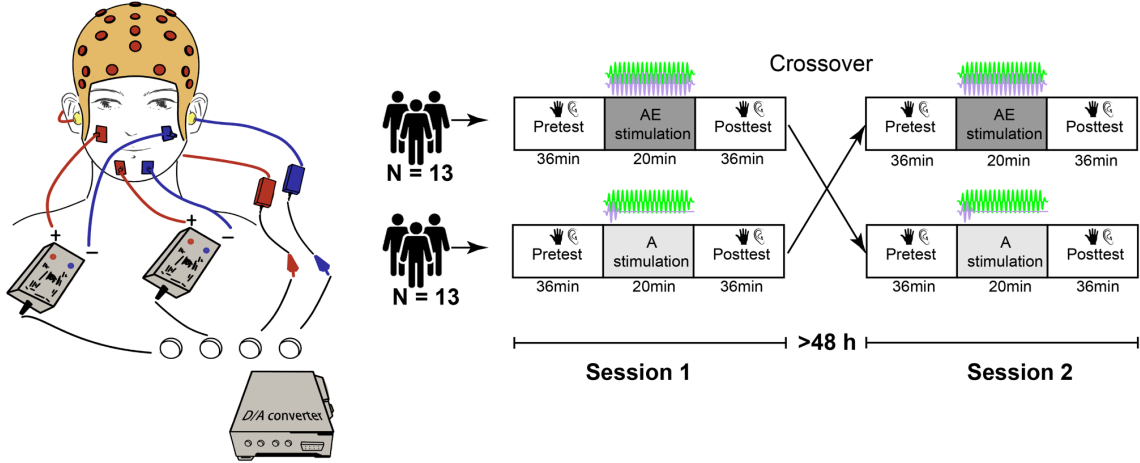
Acoustic and electric stimuli were digitally generated using a sampling rate of 16 kHz and then converted simultaneously to analog signals using a multi-channel D/A converter. In Experiment 1, phase-locked acoustic stimulation and electric stimulation were simultaneously presented for 20 min during the acoustic-electric stimulation period. During the acoustic-only stimulation period, only acoustic stimulation was presented for 20 min but the electric stimulation was slowly ramped down after the initial 30 s. The continuous presentation of acoustic stimulation ensured that participants did not receive auditory cues differentiating the two stimulation conditions. The 30-s electric stimulation at the beginning induced sensations similar to the electric stimulation in the main stimulation condition. Together these measures served to blind participants to the stimulation conditions.

Upon completion of each session, participants were asked to complete a questionnaire in which they were asked to (1) guess the time course of the delivered electric stimulation ("no stimulation", "beginning", "end", "continuous"), (2) report if they suffered any side effects, (3) report their level of familiarity with the presented music.

2.4 Experiment 2

The assessment of conscious perception in Experiment 2 was similar to that in Experiment 1, with the exceptions that only two tactile blocks were presented and only behavioral data were recorded. The stimulation administered in Experiment 2 was also similar to that in Experiment 1, except that the acoustic stimulation was removed, resulting in electric-only and electric-sham stimulation (Fig.1B).

A. Experiment 1



B. Experiment 2

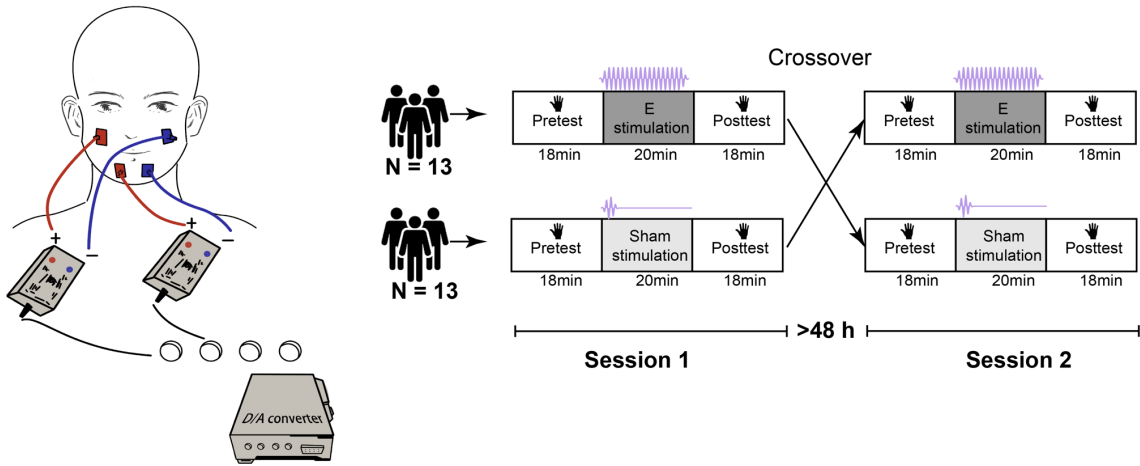


Figure 1. Stimulation approach and study design. (A). Experiment 1: Multimodal acoustic-electric TN stimulation involved the simultaneous application of music via earphones and electric current via electrodes attached to the middle and lower parts of the participant’s face (see red and blue squares). The crossover design involved two sessions separated by more than 48 hours. Each session consists of three phases: assessment of conscious perception before stimulation (pretest), application of acoustic-electric/acoustic-only stimulation, and assessment of conscious perception after stimulation (posttest). (B). Experiment 2: same as (A), but the stimulation involved electric-only and electric-sham stimulation and the assessment focused only on conscious tactile perception.

2.5 Data acquisition and processing

2.5.1 Behavior

Trials without responses were rejected and the remaining trials were classified as “detected” or “undetected” depending on the participant’s response. The detection rates were calculated as a function of stimulus intensity (from 1 to 9) and then fitted by a logistic function using a maximum likelihood criterion, as implemented in the Palamedes toolbox (Prins & Kingdom, 2018). The function is given by:

$$p = \gamma + \frac{1 - \gamma - \lambda}{1 + e^{-\beta(x-\alpha)}}$$

where γ and λ are the lower and upper bounds of the psychometric function, reflecting the guessing rate and lapse rate; α is a threshold parameter indicating the center of the psychometric function's dynamic range; β is related to the slope of the function. The guessing rate and lapse rate were fixed at zero and the other two parameters, detection threshold and slope, were set as free parameters. In this study, the threshold is the stimulation intensity yielding 50% detection rate on the fitted psychometric function and the slope is the steepness of the function at that intensity. The psychometric function was fitted separately for each participant, phase (pretest and posttest), session, and sensory task modality (tactile and auditory).

2.5.2 EEG

2.5.2.1 Recording

EEG signals were recorded during the target-detection task in Experiment 1 using 39 scalp EEG electrodes (BrainCap, Brain Products LiveAmp 64, Gilching, Germany) placed according to a standard 10–20 system. The AFz electrode was used as the ground electrode and the left mastoid electrode (A1) was used as an online reference electrode. Vertical electrooculogram was recorded by placing an extra electrode below the left eye. An additional electrocardiogram electrode was placed under the left breast to record heart activity (not analysed in the present study). Electrode impedances were kept below 10 k Ω throughout the experiment. EEG recordings were online bandpass-filtered between 0.01 and 70 Hz, and digitized with a sampling rate of 500 Hz.

2.5.2.2 Data pre-processing and ERP analysis

Data preprocessing and analysis was performed using EEGLAB 2019.1 (Delorme & 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; mean number of interpolated channels across participants: 1.0). Then, the interpolated channel data were re-referenced to the average of left and right mastoids and were band-pass filtered between 0.5 Hz and 30 Hz using a Butterworth Infinite Impulse Response (IIR) filter (zero phase shift, filter order: 6). Next, independent component analysis was performed, and artifactual components were identified and discarded (mean percentage of artifactual components: 15.4%) using the EEGLAB plugin ICLables (Pion-Tonachini, Kreutz-Delgado, & Makeig, 2019). The continuous data were segmented into epochs from -100 to 500 ms relative to stimulus onset and artifactual epochs with amplitudes exceeding ± 65 μ V were removed (mean percentage of excluded epochs: 0.8%). One participant's data were excluded from further data analysis because of excessive artifacts resulting in 11.7% of epochs removed. The artifact-free, epoched data were corrected for baseline drifts by subtracting the average amplitude from -100 to -10 ms relative to the stimulus onset from the epoch.

To control for stimulation-related confounds (i.e., number of epochs, stimulation intensity) in the analysis, we balanced the number of epochs between detected and undetected conditions per intensity level using subsampling in the condition with a larger number of epochs. Compared to randomly selecting an equal number of epochs, this method was designed to minimize the interval difference (i.e., the presentation interval) between the conditions (i.e., detected vs. undetected), and control for the time-related confounds (e.g., fatigue and adaptation). One participant was excluded from further data analysis due to a limited number of epochs left (13.3%) after balancing. Overall, the average number of retained tactile and auditory epochs was 118 and 140, respectively, with the greatest number of epochs near the detection threshold as expected (Fig.2C). Finally, an equal number of detected and undetected epochs were averaged in the time domain, creating time-locked ERPs for each electrode, each condition (detected and undetected), each modality (tactile and auditory) and each participant.

ERP components (and corresponding time windows for analysis) were chosen based on previous research, with particular focus on an early negative component (tactile: 125-180ms; auditory: 120-200ms) and a late positive component (250-500ms) related to conscious perception (Dembski et al., 2021). Mean amplitudes were calculated using time-window averages at each channel, separately for detected and undetected trials. To find the channels showing significant differences related to conscious perception, the amplitudes for detected and undetected trials were compared per channel with paired t-tests and the false-discovery rate (FDR) was controlled to correct for multiple comparisons. Channel clusters that were found to show significant differences between detected and undetected trials in each phase and each session were defined as regions of interest (ROIs). Finally, difference (detected minus undetected) waves were computed for each phase and each session, and the amplitudes of the early and late ERP components were averaged across the aforementioned time windows and the ROIs.

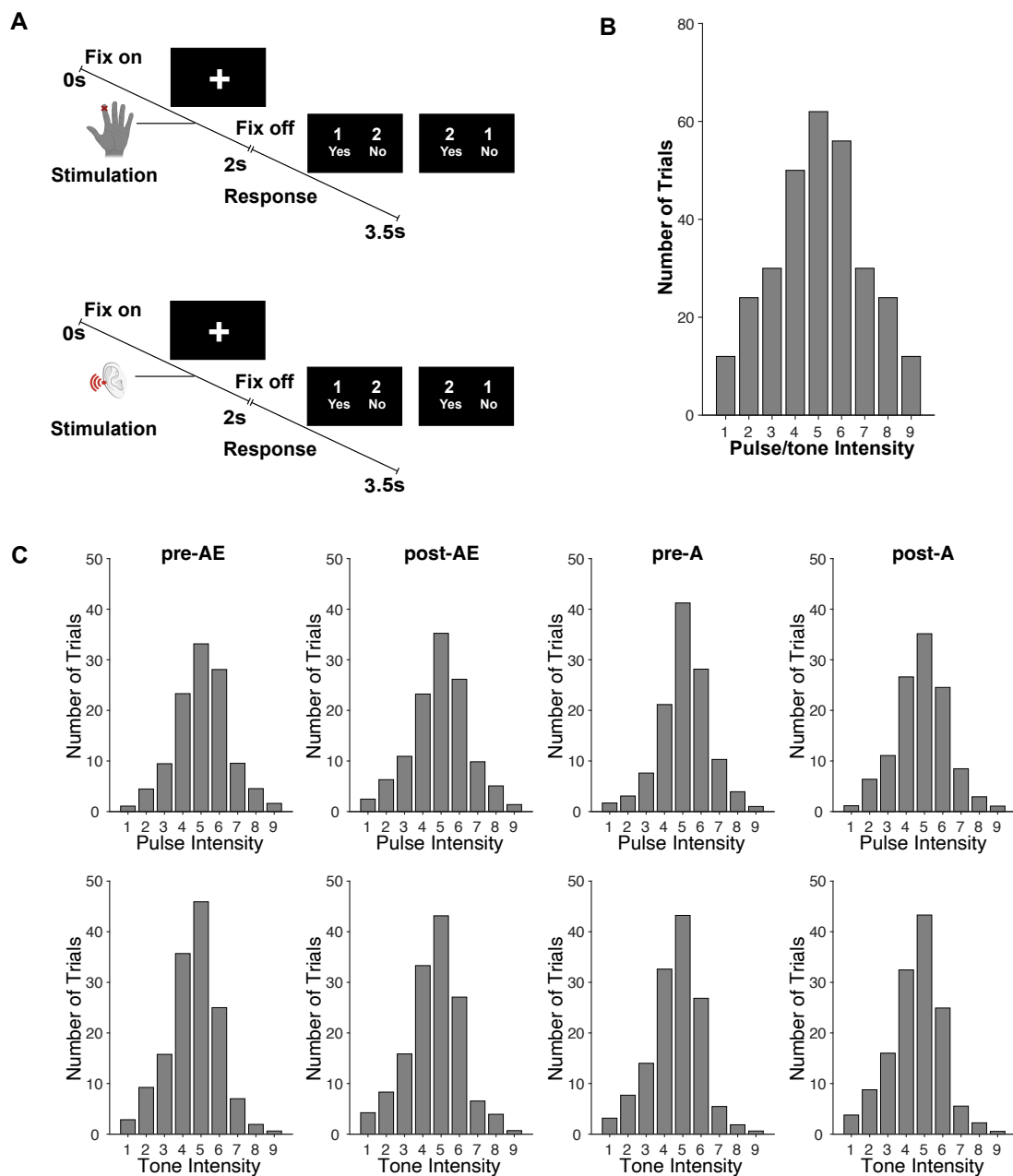


Figure 2. Trial design and trial distributions. (A). Schematic of an exemplary tactile-detection trial (upper) and an auditory-detection trial (bottom) presented in different blocks. A trial started with the presentation of a fixation cross on the screen. A tactile or auditory stimulus followed with a random delay 1000-1500 ms after the fixation onset. Next, a 1500-ms response screen was displayed, instructing participants to respond whether they had detected a stimulus or not. (B). Intensity distribution of the presented trials. The number of trials across intensity levels followed a normal distribution, with a maximal number of trials at the threshold level (intensity 5). (C). Intensity distribution of trials after subsampling for the neural data analysis. The subsampling resulted in a matched number of detected and undetected trials, for each intensity.

2.6 *Dynamic causal modeling*

DCM is an effective connectivity analysis in which sensor-level data (here: ERPs) are fitted with a generative model of observed responses (David, Harrison, & Friston, 2005). The model consists of two main components: a neuronal model, describing dynamics of neural activity in a network comprising effective (directional) connectivity parameters between network nodes (brain regions), and an observation model, mapping hidden states (neural activity estimates) to sensor-level measurements. Models are fitted to the data using variational Bayesian methods (Kiebel, Garrido, Moran, Chen, & Friston, 2009) to obtain estimates of log-model evidence (enabling a comparison between alternative models) and model parameters (e.g., connectivity estimates). The connectivity parameters comprise baseline estimates shared across experimental conditions ("A matrix"), modulatory parameters denoting connectivity changes due to experimental conditions ("B matrix"; e.g., stimulus detection), and input parameters ("C matrix", defining network nodes receiving sensory inputs). A detailed formulation of DCM for ERPs and its application to tactile detection can be found in a previous study (Aukstulewicz & Blankenburg, 2013). In the present study, DCMs were used to identify (1) a neural network model explaining ERP differences to detected and undetected stimuli (i.e., modeling conscious perception effects) and (2) connection changes in the model induced by the acoustic-electric stimulation. This analysis was conducted using the SPM 12 toolbox (<http://www.fil.ion.ucl.ac.uk/spm/>).

The DCMs were fit to the stimulus-evoked ERPs from 1 to 500 ms peristimulus time using an ERP convolution-based neural mass model (Moran, Pinotsis, & Friston, 2013). Based on findings from a related study using DCM (Aukstulewicz & Blankenburg, 2013), the contralateral primary somatosensory cortex (cSI), bilateral secondary somatosensory cortex (cSII, iSII) and bilateral premotor cortex (cPMC, iPMC) were defined as sources constituting network nodes. The locations (MNI coordinates) of the five sources were obtained from previous studies and were as follows: cSI (52, -22, 44), cSII (52, -16, 16), iSII (-58, -20, 16), cPMC (30, -20, 54), iPMC (-30, -20, 54) (Aukstulewicz & Blankenburg, 2013; Wacker, Spitzer, Lützkendorf, Bernarding, & Blankenburg, 2011).

Firstly, the basic DCM architecture ("A matrix") was identified using the ERPs evoked by both detected and undetected tactile stimuli. Five alternative models were constructed differing in the number of sources and the extrinsic connections between them (Fig.6A). Each model was fitted separately for each participant, resulting in 5×26 models in pretest and posttest. These models were then compared using fixed-effects BMS, which resulted in the selection of a winning model that fitted the data best across all participants (Stephan et al., 2010).

Secondly, the selected winning model was optimized with respect to the condition-dependent changes ("B matrix") in extrinsic connections to explain the observed tactile ERP difference between detected and detected trials. Six models were constructed differing with respect to their extrinsic connections (Fig.6C). Three models assumed modulatory connections between cSI and cSII/iSII and the other three models added modulatory connections between SII and PMC. The exogenous tactile

information is modeled as a direct input entering cSI ("C matrix"). As above, each model was fitted separately for each participant, and Bayesian model selection (BMS) was used to select the winning model.

To estimate the changes of model parameters caused by acoustic-electric stimulation, a second-level analysis was conducted using parametric empirical Bayes (PEB) (Rosch, Auksztulewicz, Leung, Friston, & Baldeweg, 2019; Zeidman et al., 2019). PEB is used to estimate commonalities and differences in model parameters at the group level, modelled as a second-level design matrix (typically, under the assumption that parameters are normally distributed in the participant sample). Our PEB design matrix contained two columns: the first column represented the group mean (i.e., 1), and the second column represented time (i.e., -1 for pretest, 1 for posttest). The winning model from the previous step was entered per participant and time into the PEB, and subjected to Bayesian model reduction (BMR) and averaging (BMA) to prune away model parameters that did not contribute to the model evidence. To enable statistical inference, we retained model parameters at a posterior probability higher than 0.99 (i.e., strong evidence for a change of the parameters induced by the acoustic-electric stimulation).

2.7 Statistical analysis

To test for successful participant blinding, Fisher's exact test was run to determine if the perceived stimulation type differed between two stimulation sessions in both experiments. To assess the effect of stimulation on behavioral and neural measures within each stimulation session, two-sided paired t-tests were used. To test whether the effect differed between stimulation sessions, a two-way repeated-measures ANOVA with two within-subjects factors "*stimulation*" (acoustic-electric vs. acoustic-only in Experiment 1; electric-only vs. electric-sham in Experiment 2) and "*time*" (pretest vs. posttest) was used. The correlation between behavioral and neural effects of stimulation and its significance were assessed using Pearson's correlation coefficient *R*. A significance criterion $\alpha = 0.05$ was used and type-I error probabilities inflated by multiple comparisons were corrected by controlling the FDR.

3 Results

In both experiments, participants' subjective reports of the received electric stimulation did not differ between the two experimental sessions, which involved respectively verum and sham electric stimulation ($p < 0.05$, Fisher's exact test). This suggests that participants could not reliably distinguish between the presence and absence of the electric stimulation. No participant reported any side effect. Two participants reported that they were familiar with the music (i.e., the acoustic stimulation) in both sessions (familiarity scores: 3 and 4 out of 5), while all other participants reported they were completely unfamiliar with the music (familiarity score: 0 out of 5).

3.1 Acoustic-electric stimulation but not acoustic-only stimulation modulates conscious tactile perception

To assess whether acoustic-electric stimulation or acoustic-only stimulation can improve subsequent conscious perception, we compared stimulus-detection thresholds (interpreted as response criterion, as e.g. a leftward shift of the psychometric curve represents a bias towards reporting "detected" (Gold & Ding, 2013; Kar & Krekelberg, 2014; Ruzzoli, Marzi, & Miniussi, 2010)) and psychometric slopes (interpreted as perceptual sensitivity, as e.g. steeper slope represents higher sensitivity (Gold & Ding, 2013; Parker & Newsome, 1998; Zazio, Bortoletto, Ruzzoli, Miniussi, & Veniero, 2019)) on tactile and auditory target-detection tasks after vs before stimulation in Experiment 1 (Fig.1A). We found a

significant decrease in detection threshold, but not in slope, in the tactile task after vs before acoustic-electric stimulation (threshold: $t_{25} = 3.936$, $p = 5.8 \times 10^{-4}$, effect size $d = 0.772$; slope: $t_{25} = -0.061$, $p = 0.952$, $d = -0.012$, Fig.3A-C). In contrast, we found no significant change in either detection threshold or slope in the tactile task after vs before acoustic-only stimulation (threshold: $t_{25} = 1.831$, $p = 0.079$, $d = 0.359$; slope: $t_{25} = 1.030$, $p = 0.313$, $d = 0.202$, Fig.3A-C). This observation of a behavioral gain in tactile perception after acoustic-electric stimulation (vs. acoustic-only stimulation) was statistically supported by results from a two-way repeated-measures ANOVA, which revealed a significant *stimulation* (acoustic-electric stimulation versus acoustic-only stimulation) \times *time* (pretest versus posttest) interaction ($F_{1,25} = 8.572$, $p = 0.007$, effect size = 0.255). For conscious auditory perception, we found no significant change in detection threshold or slope after vs before acoustic-electric stimulation (threshold: $t_{25} = 0.707$, $p = 0.707$, $d = 0.075$; slope: $t_{25} = 0.500$, $p = 0.621$, $d = 0.098$) or acoustic-only stimulation (threshold: $t_{25} = -1.123$, $p = 0.272$, $d = -0.220$; slope: $t_{25} = -0.123$, $p = 0.903$, $d = -0.024$) (Fig.3D-F). These results indicate that (1) acoustic-electric stimulation, but not acoustic-only stimulation, improved subsequent conscious tactile perception, and this improvement affected exclusively participants' response criterion, not perceptual sensitivity; (2) acoustic-electric stimulation and acoustic-only stimulation did not systemically modulate subsequent conscious auditory perception.

To investigate neural responses reflecting conscious perception, we computed ERPs evoked by identical stimuli that participants detected vs. undetected. Channel-by-channel analysis revealed that detected (vs. undetected) tactile stimuli elicited a larger negativity distributed in the contralateral temporal area, which was defined as a ROI (Fig.S1A). The detected (vs. undetected) auditory stimuli evoked a similar negativity distributed in the frontal and central scalp areas (Fig.S1B). Detected stimuli in both modalities evoked a large positivity distributed widely over the scalp covering mainly centroparietal areas (Fig.S1). ERP waveforms averaged over the aforementioned ROIs are depicted in Fig.S2. Consistent with previous research (Al et al., 2020; Eklund & Wiens, 2019), the early negativity evoked by the detected stimuli was observed at ~140 ms from the target onset, and a later more sustained positivity was observed 250-500 ms from the target onset (Fig.S2). These components were reliably observed across phases (pretest, posttest) and sessions. Next, an awareness-related difference waveform was obtained by subtracting the responses to undetected stimuli from the responses to detected stimuli.

To assess whether acoustic-electric stimulation or acoustic-only stimulation enhanced neural responses reflecting conscious perception, we compared the awareness-related difference waveforms (detected minus undetected) after vs before stimulation. We found a significant increase in the amplitude of the late tactile component, but not the early component, after vs before acoustic-electric stimulation (late component: $t_{25} = -3.875$, $p = 6.8 \times 10^{-4}$, $d = -0.760$; early component: $t_{25} = -0.297$, $p = 0.769$, $d = -0.058$; Fig.4A-D). However, we found no significant change in the amplitude of either early or late tactile component after vs before acoustic-only stimulation (late component: $t_{25} = -0.279$, $p = 0.782$, $d = -0.055$; early component: $t_{25} = -0.628$, $p = 0.536$, $d = -0.123$; Fig.4A-D). Analogous to the behavioral analysis, the different effects of acoustic-electric and acoustic-only stimulation on the amplitude of the late tactile component were confirmed by a significant *stimulation* (acoustic-electric stimulation versus acoustic-only stimulation) \times *time* (pretest versus posttest) interaction ($F_{1,25} = 5.002$, $p = 0.034$, effect size = 0.167). For conscious auditory perception, we found no significant change in the amplitude of early or late auditory component after vs before acoustic-electric stimulation (late component: $t_{25} = -0.489$, $p = 0.629$, $d = -0.096$; early component: $t_{25} = 0.285$, $p = 0.778$, $d = 0.056$) or acoustic-only stimulation (late component: $t_{25} = 0.133$, $p = 0.896$, $d = 0.026$; early component: $t_{25} = -0.951$, $p = 0.351$, $d = -0.187$) (Fig.4E-H). These results indicate that (1) acoustic-electric stimulation but not acoustic-only stimulation enhanced subsequent neural responses to conscious tactile perception and the effect applied exclusively to the late but not the early response; (2) acoustic-electric stimulation and acoustic-only stimulation did not systemically modulate subsequent neural responses to conscious auditory perception.

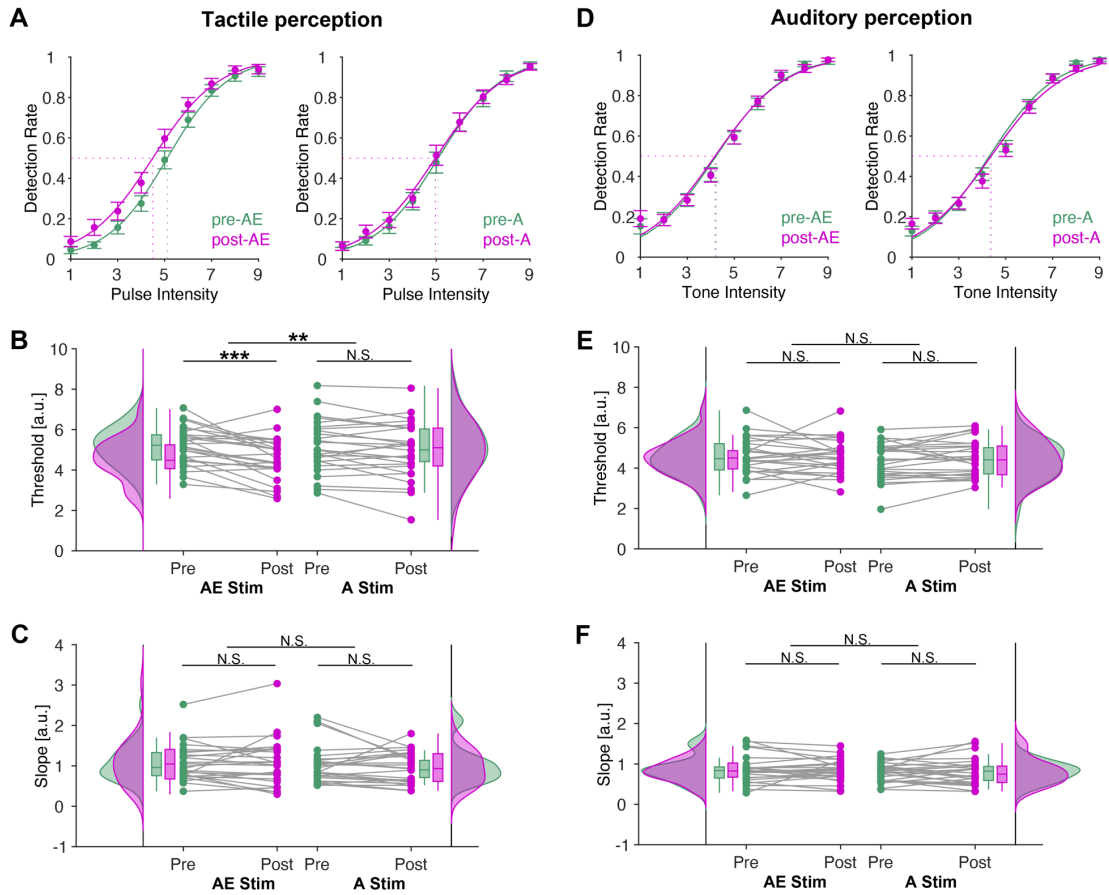


Figure 3. Behavioral performance in the target-detection task before and after acoustic-electric stimulation and acoustic-only stimulation. (A). Group-averaged psychometric function in the tactile-detection task. (B). The left and right pairs of plots respectively represent the tactile-detection threshold before and after acoustic-electric stimulation (left) and acoustic-only stimulation (right). The tactile threshold decreased significantly after acoustic-electric stimulation, but not after acoustic-only stimulation. (C). Same as (B), but for the psychometric slope. No significant effect on psychometric slope was observed after acoustic-electric stimulation or acoustic-only stimulation. (D-F). Same as (A-C), but for behavioral performance in the auditory-detection task. No significant effect of acoustic-electric stimulation or acoustic-only stimulation on the auditory-detection threshold or slope was observed. Data are presented as mean \pm sem across participants in (A, D). The raincloud plots in (B-C, E-F) visualize the data distribution, the horizontal line within each boxplot indicates the median value across participants; 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. The dots in (B-C, E-F) represent individual participants. Green and magenta respectively represent pretest and posttest. N.S. non-significant, ** $p < 0.01$, *** $p < 0.001$.

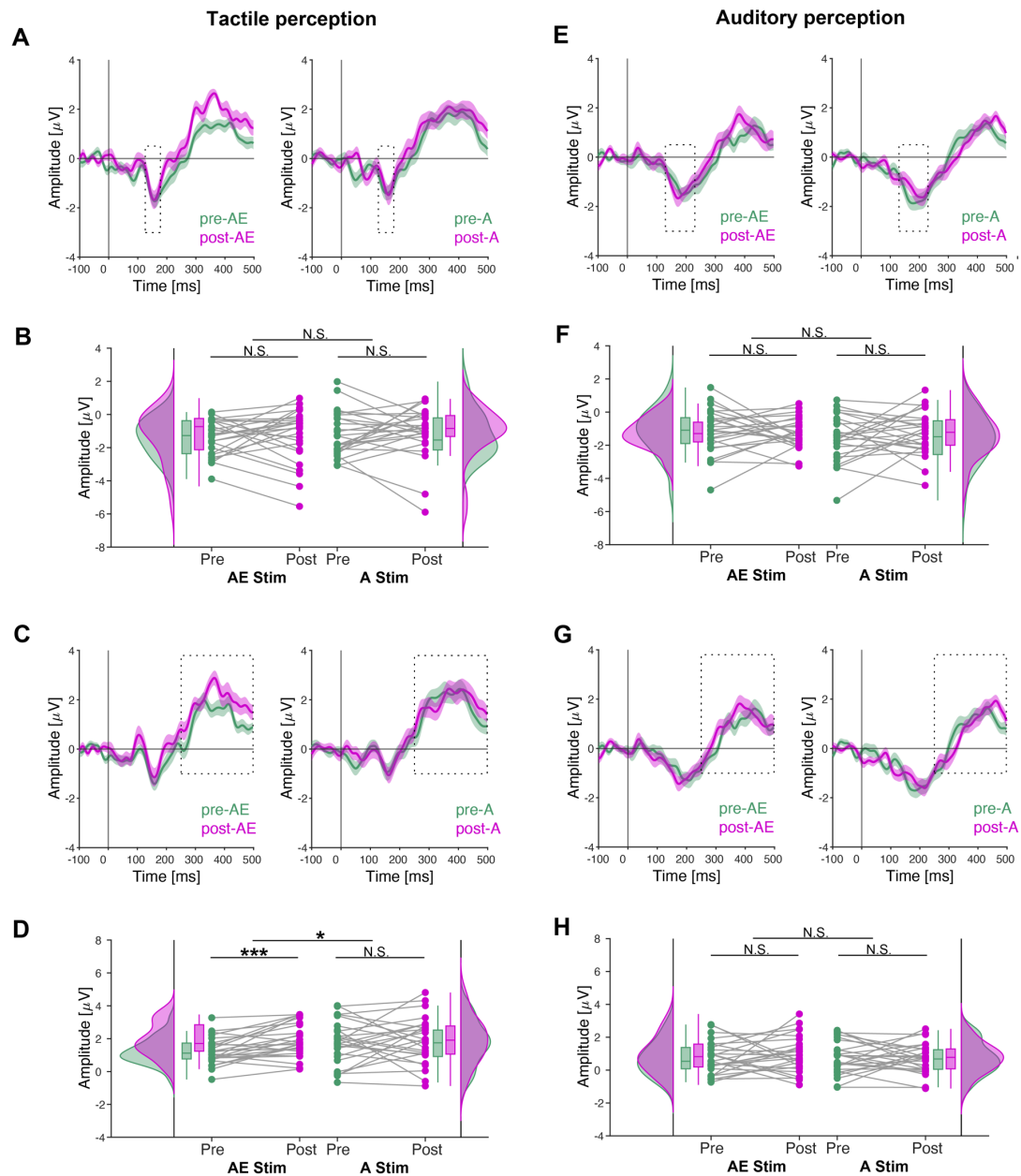


Figure 4. Neural responses in the target-detection task before and after acoustic-electric stimulation and acoustic-only stimulation. (A). Group-averaged tactile awareness-related difference waveforms of the early tactile component (averaged over ROIs). (B). The left and right pairs of plots respectively represent the amplitude of the early tactile component before and after acoustic-electric stimulation (left) and acoustic-only stimulation (right). No significant effect of either stimulation was observed on the early component. (C-D). Same as (A-B), but for the late tactile component. The amplitude of the late tactile component increased after acoustic-electric stimulation, but not after acoustic-only stimulation. (E-H). Same as (A-D), but for neural responses in the auditory-detection task. No significant effect of either stimulation on the early or late auditory component was observed. Data are presented as mean \pm sem across participants in (A, C, E, G). The raincloud plots in (B, D, F, H) visualize the data distribution, the horizontal line within each boxplot indicates the median value across participants; 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. The dots represent individual participants. Green and magenta respectively represent pretest and posttest. N.S. non-significant, * $p < 0.05$, *** $p < 0.001$.

3.2 No significant effect of electric-only stimulation on conscious tactile perception

While the results from Experiment 1 suggest that acoustic-electric stimulation, but not acoustic-only stimulation, can improve conscious tactile perception, it remains unclear whether the effect is caused by the electric stimulation alone or its interplay with the acoustic stimulation. To address this question, we performed Experiment 2 in which we applied electric-only and electric-sham stimulation (Fig.1B, see more details in Methods) and compared tactile-detection thresholds and psychometric slopes after vs before stimulation. We found no significant change in tactile-detection threshold or slope after vs before electric-only (threshold: $t_{25} = 1.490$, $p = 0.149$, $d = 0.292$; slope: $t_{25} = -0.694$, $p = 0.494$, $d = -0.136$) or electric-sham stimulation (threshold: $t_{25} = 0.179$, $p = 0.859$, $d = 0.035$; slope: $t_{25} = 1.260$, $p = 0.219$, $d = 0.247$) (Fig.5). This result suggests that prior electric-only stimulation and/or placebo-related changes do not systemically modulate conscious tactile perception. Taken together, these results indicate that the improvement of conscious tactile perception observed in Experiment 1 was caused by the interplay of acoustic-electric stimulation, rather than by the acoustic stimulation alone (Experiment 1), the electric stimulation alone, or any placebo-related change (Experiment 2).

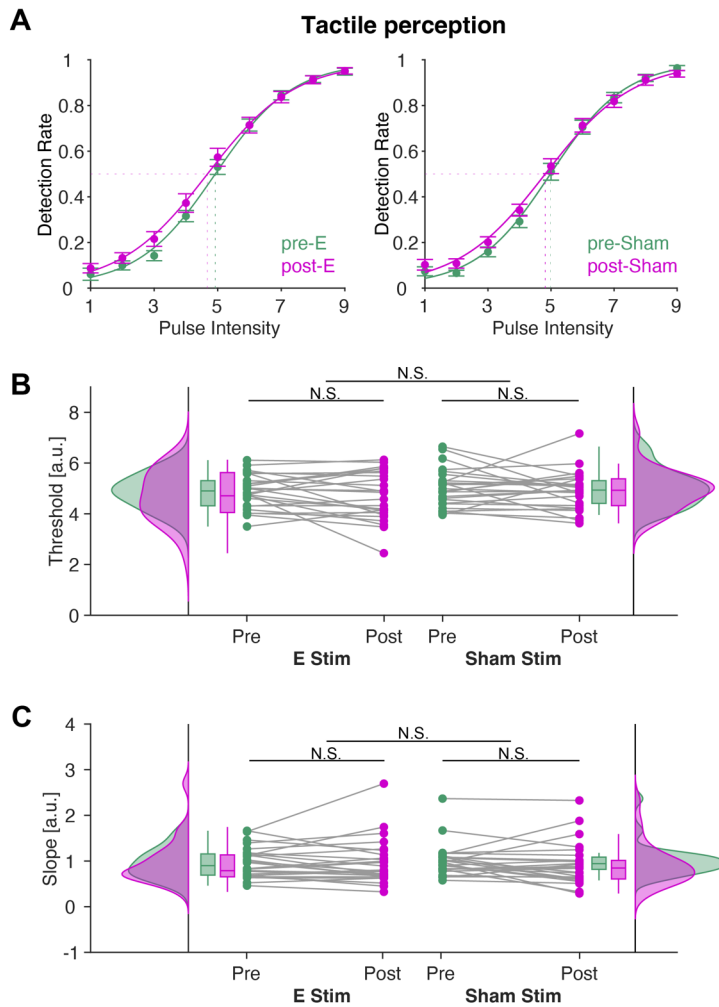


Figure 5. Behavioral performance in the tactile-detection task before and after electric-only stimulation and electric-sham stimulation. (A). Group-averaged psychometric function in the tactile-detection task. (B). The left and right pairs of plots respectively represent the tactile-detection threshold before and after electric-only stimulation (left) and electric-sham stimulation (right). (C). Same as (B), but for the psychometric slope. No significant effect of electric-only stimulation or electric-sham stimulation on tactile-detection threshold or slope was observed. Data are presented as mean \pm sem across participants in (A). The raincloud plots in (B-C) visualize the data distribution, the horizontal line within each boxplot indicates the median value across participants; 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. The dots in (D-C) represent individual participants. Green and magenta respectively represent pretest and posttest. N.S. non-significant.

3.3 *Acoustic-electric stimulation modulates connections in a recurrent neural processing model*

To explore potential neural mechanisms underlying the observed effects of acoustic-electric stimulation on tactile perception, we conducted a DCM analysis of tactile neural responses in the following three steps. Firstly, five models differing in the number of sources and the extrinsic connections between these sources were fitted to the tactile-evoked response (Fig.6A). Using BMS, we found decisive evidence for a model in both pretest and posttest (log-model evidence for the winning model, relative to the second-best model: pretest, 3173; posttest, 2225; both corresponding to an exceedance probability of >99%) that included reciprocal connections between cSI and cSII, and between bilateral SII and bilateral PMC (Fig.6B, model 4). Secondly, six models allowing for changes in the modulatory connectivity were fitted to explain the tactile-response difference between detected and undetected stimuli (Fig.6C). A model incorporating global recurrent connections showed the greatest evidence (log-model evidence relative to the second-best model: pretest, 3223; posttest, 1570; exceedance probability >99%) and was therefore selected as the winning model (Fig.6D, model 6). This result is in accordance with the previous study that identified a role of recurrent neural processing in conscious tactile perception (Auksztulewicz & Blankenburg, 2013). Thirdly, the winning model for each subject before and after stimulation was combined into a single PEB model. Connection changes induced by the acoustic-electric stimulation were identified and calculated using BMR and BMA. We found that the acoustic-electric stimulation had an excitatory effect on the connections from cSI to cSII (posterior estimate (Ep) = 0.301, a log-scaling parameter corresponding to the connection change modulated by acoustic-electric stimulation; posterior probability (Pp) = 1), from iPMC to iSII (Ep = 0.389; Pp = 1), and from iPMC to cPMC (Ep = 0.665; Pp = 1). Moreover, the acoustic-electric stimulation had an inhibitory effect on the connection from iPMC to cSII (Ep = -0.271; Pp = 1) (Fig.7A). These results indicate that the acoustic-electric stimulation modulated connection strength within a global recurrent processing model.

To assess whether the modulatory effects of acoustic-electric stimulation on connection were related to the observed improvements of conscious tactile perception, we next explored the correlation between connection changes (i.e., posterior estimates) and tactile-threshold changes after acoustic-electric stimulation. We found a significant negative correlation between the connection change from cSI to cSII and the change of tactile-detection threshold ($R = -0.473$, $p = 0.029$, FDR corrected). We obtained a similar result for the connection change from iPMC to cSII ($R = -0.405$, $p = 0.040$, FDR-corrected) (Fig.7B). These results suggest that stronger improvements of conscious tactile perception were accompanied by increased bottom-up excitation from cSI to cSII and reduced top-down inhibition from iPMC to cSII.

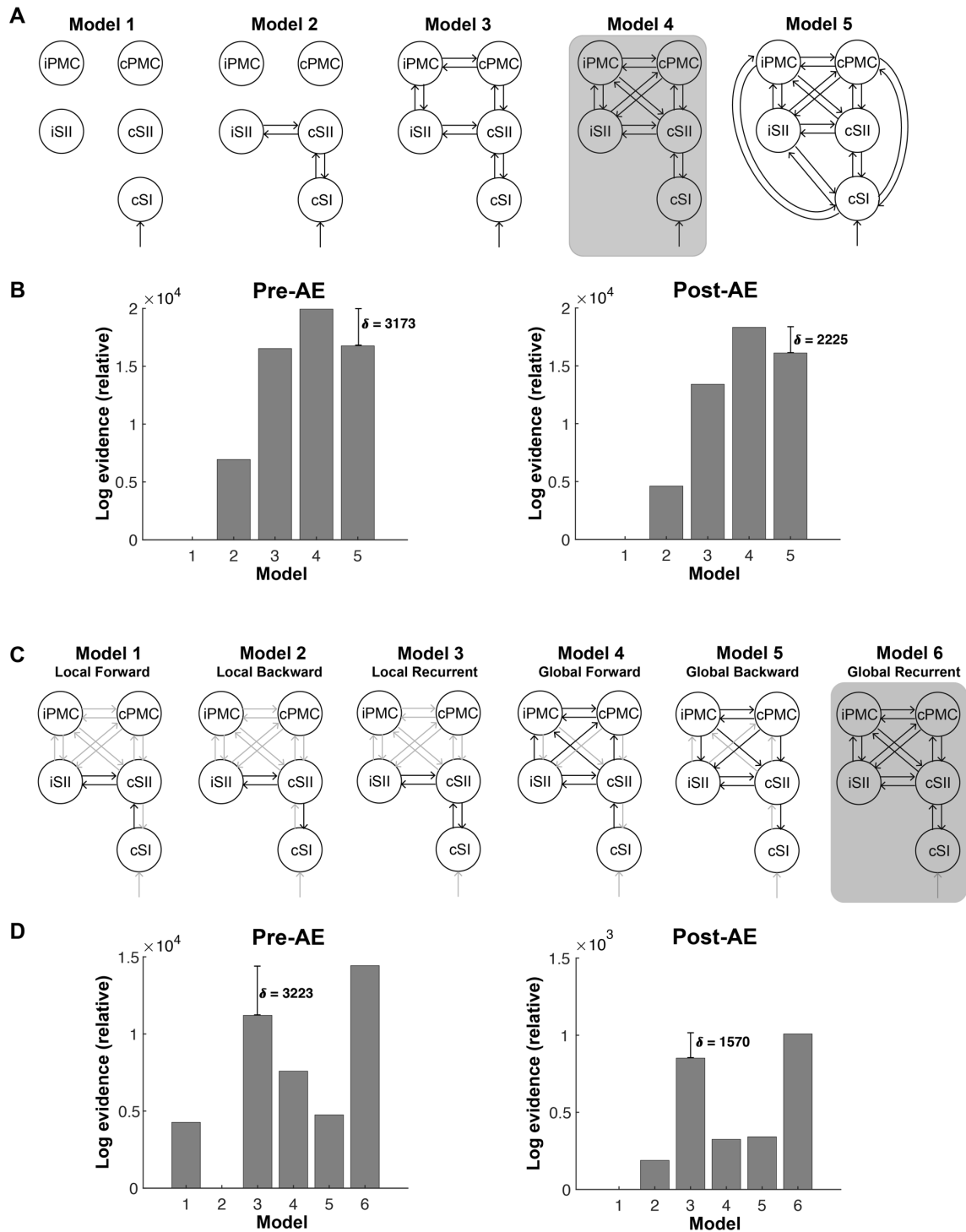


Figure 6. Model space of dynamic causal modeling. (A). Five alternative models of effective connectivity were fitted to individual participants' ERPs evoked by the tactile stimulation. All models included the tactile input to the contralateral SI and differed with respect to the number of sources and the extrinsic connections between these sources. (B). Fixed-effects Bayesian model selection revealed that model 4 (shaded dark gray) outperformed the other models. Model 4 included five sources (cSI, cSII, iSII, cPMC, iPMC) and recurrent connections between cSI and cSII, and between SII and PMC. (C). Modeling the modulatory effect of acoustic-electric stimulation on extrinsic connections. Six models were designed in which stimulation modulated a different subset of extrinsic connections between cSI and cSII, and between bilateral SII and bilateral PMC. (D). Fixed-effects Bayesian model selection revealed that model 6 (i.e., global recurrent model, shaded dark gray) outperformed the

other models. Model 6 allowed for recurrent connections among the five sources to be modulated by acoustic-electric stimulation.

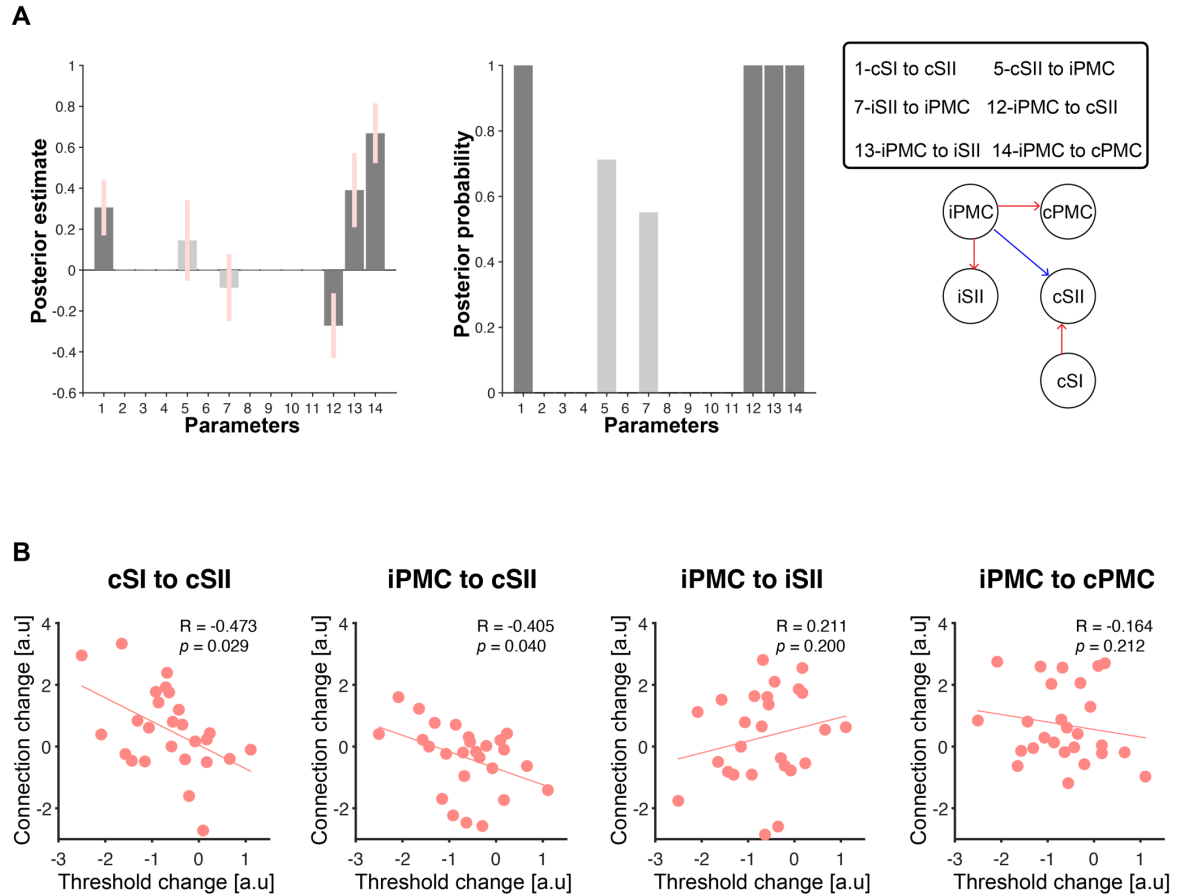


Figure 7. Effects of acoustic-electric stimulation on model parameters estimated using PEB. (A). Posterior estimates of connections (left bar plot) as log-scaling values relative to the priors and posterior probability of these connections (right bar plot). Significant changes (posterior probability > 99%) induced by acoustic-electric stimulation were observed in a subset of connections (right network graph) as the posterior estimates were significantly greater or smaller than the prior mean of zero (i.e., a log scaling of 100%): an excitation of connections between cSI and cSII, iPMC and iSII, and iPMC and cPMC (red arrows), and an inhibition of connections from iPMC to cSII (blue arrows). (B). The scatterplot shows results from a correlation analysis testing for an association between changes in tactile-detection threshold and changes in connectivity parameter. Correlation coefficient R and p -value describe, respectively, the strength and statistical significance of the coupling (linear regression line) across all participants.

4 Discussion

We found that (1) acoustic-electric stimulation can lead to an improvement of conscious tactile perception without a concomitant change in auditory perception in healthy human participants, (2) this improvement is caused by the interplay of acoustic and electric stimulation rather than any of the unimodal stimulation alone, and (3) the effect of acoustic-electric stimulation on consciousness

correlates with inter-regional connection changes in a recurrent neural processing model. Overall, our findings provide evidence that multimodal acoustic-electric stimulation can modulate conscious tactile perception and propose modulation of inter-regional connections as a potential neural mechanism.

4.1 *Acoustic-electric stimulation modulates conscious tactile perception*

We found that prior acoustic-electric stimulation improved conscious tactile perception, as indicated by increases in both detection performance and the awareness-related ERP component after vs before acoustic-electric stimulation. These observations confirm and replicate previous findings in DOC patients, showing that acoustic-electric stimulation can reliably promote consciousness (Wu et al., 2022).

Gamma oscillations have been previously associated with conscious tactile perception in both observational and experimental studies (Gross, Schnitzler, Timmermann, & Ploner, 2007; Meador, Ray, Echauz, Loring, & Vachtsevanos, 2002; Siegle, Pritchett, & Moore, 2014). For example, Siegle et al. optogenetically modulated gamma activity in mice and found improved detection of weak tactile stimulation (Siegle et al., 2014), thereby shedding light on a causal role of gamma oscillations in conscious tactile perception. Therefore, the improvements of conscious tactile perception observed in our study may be attributed to stronger gamma oscillations induced by the application of the acoustic-electric stimulation, which indeed fluctuated strongly at gamma frequency (see Methods). Although gamma oscillations were not measured in this study, it has been well-established that rhythmic stimulation can entrain rhythmic brain oscillations at the exact frequency of the applied stimulation. For example, our previous study has shown an increase of neural activity in the gamma band after multimodal acoustic-electric stimulation in the gamma band (Wu et al., 2022).

Interestingly, we found that acoustic-electric stimulation affected exclusively participants' response criterion, not perceptual sensitivity to tactile stimulation. This finding is compatible with prior work showing effects of transcranial magnetic stimulation in gamma band on response criterion, but not perceptual sensitivity, in a visual-detection task (Chanes, Quentin, Tallon-Baudry, & Valero-Cabre, 2013). The researchers proposed that gamma oscillations reflect sensory evidence regardless of the stimulus presence and can therefore account for response bias (Chanes et al., 2013; Riddle, Hwang, Cellier, Dhanani, & D'Esposito, 2019). Thus, it is conceivable that our application of gamma acoustic-electric stimulation induced gamma oscillations that reflected accumulating sensory evidence of tactile stimulation input, which then resulted in the observed shift of response criterion.

Similarly, we found acoustic-electric stimulation enhanced exclusively the late but not the early neural response to detected vs. undetected targets. The observation of significant ERP components that discriminate between detected vs. undetected stimuli in an early and a late time window is consistent with previous ERP studies on awareness (Al et al., 2020; Auksztulewicz & Blankenburg, 2013; Auksztulewicz et al., 2012; Eklund, Gerdfeldter, & Wiens, 2020; Eklund & Wiens, 2019). The early negative component has been associated with perceptual sensitivity and is thought to be unaffected by response criterion (M. Koivisto & Grassini, 2016; Mazzi, Mazzeo, & Savazzi, 2020). As such, the lack of a significant stimulation effect on the early component in the present study is consistent with the null result on perceptual sensitivity. However, there is an ongoing controversy on whether the late component is a reliable correlate of consciousness or reflects post-perceptual processes (Cohen, Ortego, Kyroudis, & Pitts, 2020; Forster, Koivisto, & Revonsuo, 2020). Recent research has shown that the late component may not emerge in response to perceived yet unreported stimuli (stimuli in that study were irrelevant to the participants' task) (Pitts, Metzler, & Hillyard, 2014). Similarly, Schroder et al. reported that the late component may not emerge from target detection when report requirements are controlled using a matching task (Schroder, Nierhaus, & Blankenburg, 2021). Thus, our interpretation of the stimulation-induced increase in the late positive component as an effect on late conscious processing (as reflected by) needs to be treated with caution. It remains

possible that the acoustic-electric stimulation modulated the post-perceptual reporting rather than conscious tactile perception *per se*.

4.2 No effect of acoustic-electric stimulation on conscious auditory perception

We found that acoustic-electric stimulation did not systemically modulate subsequent conscious auditory perception. Even though a few studies have shown that transcranial alternating current stimulation (tACS) can directly modulate auditory perception in a phase-dependent manner (Neuling, Rach, Wagner, Wolters, & Herrmann, 2012; Riecke, Formisano, Herrmann, & Sack, 2015), the evidence for overall or sustained benefits from tACS (compared with sham stimulation) for conscious auditory perception is scarce. For example, Riecke et al. found that the relative phase of 4-Hz tACS modulated the detection of 4-Hz click trains; however, click detection did not differ significantly under 4-Hz tACS vs. sham stimulation (Riecke et al., 2015).

A recent study revealed that the effects of gamma tACS on auditory temporal perception may depend on the difference between the stimulation frequency and the endogenous brain oscillation frequency. More specifically, tACS with frequencies 4 Hz above the endogenous gamma frequency was found to accelerate the endogenous oscillation and improve auditory gap-detection performance. In contrast, tACS with frequencies 4 Hz below the endogenous frequency did not improve auditory perception (Baltus, Wagner, Wolters, & Herrmann, 2018). The endogenous gamma frequency has been consistently reported to exceed 45 Hz in many human studies (Baltus & Herrmann, 2015; Zaehle, Rach, & Herrmann, 2010). In light of this, the gamma frequency applied in our study (40 Hz) may be lower than the typical endogenous oscillation frequency, which may explain why it did not improve auditory perception in our study. However, our interpretation requires caution, given the task differences across the studies (i.e., tone detection in our study vs. gap detection in Baltus et al.'s study).

4.3 No effect of unimodal stimulation on conscious tactile perception

We found that prior acoustic-only or electric-only stimulation and/or potential placebo-related changes did not systemically modulate conscious tactile perception, suggesting that the observed improvement of conscious tactile perception after acoustic-electric stimulation was caused by the interplay of the acoustic and electric stimulation, rather than by the unimodal acoustic or electric stimulation alone. These findings are in line with the studies in tinnitus and AD that showed superior effects of multimodal stimulation over unimodal stimulation (see Introduction).

One potential explanation could be that the unimodal acoustic and electric stimulation have simple additive effects. However, this interpretation is difficult to reconcile with our observation of non-significant effects of either unimodal acoustic stimulation or unimodal electric stimulation, rendering this alternative less plausible. Another, perhaps more intriguing explanation is that the consciousness benefits were driven by the integration of the acoustic and electric inputs. Indeed, this notion has been supported by animal studies. For example, auditory-tactile integration has been found in the primary auditory cortex of monkeys (Lakatos, Chen, O'Connell, Mills, & Schroeder, 2007). Moreover, animal studies in mice have shown that auditory stimulation and forepaw tactile stimulation activate neurons in the somatosensory cortex. When the tactile stimulation was paired with the auditory stimulation, the neuronal activation in somatosensory cortex and behavioral performance in a tactile-based task were significantly improved. These results indicate that the somatosensory cortex can encode multisensory information and auditory input to it can enhance its sensory encoding, leading to changes in responses to tactile input (Godenzini et al., 2021). Although we could not directly extract participants' somatosensory cortical activity, our DCM results suggest a neuronal state change in the somatosensory cortex after acoustic-electric stimulation, which we discuss further below.

Our null results based on unimodal electric stimulation are consistent with a recent tACS study that applied tACS over SI at alpha, beta, and gamma frequencies and found no significant change in near-threshold tactile perception and tactile temporal discrimination threshold (Manzo et al., 2020). However, it remains possible that unimodal stimulation did not reach the threshold for promoting consciousness. Future research may examine this possibility by e.g. applying the stimulation at an increased intensity.

4.4 *Acoustic-electric stimulation modulates connections in a recurrent neural processing model*

Our finding that a recurrent neural processing model can account for cortical responses reflecting conscious tactile perception aligns well with results from a prior study (Auksztulewicz & Blankenburg, 2013). We found that acoustic-electric stimulation had a modulatory effect on the following inter-regional connections within the recurrent processing model: from cSI to cSII, from iPMC to iSII, and from iPMC to cPMC, and from iPMC to cSII. These findings suggest that the acoustic-electric stimulation affected both somatosensory areas and PMC, and modulated the strength of the direct connections between them. However, it remains possible that the observed alterations in inter-regional cortical connections may be driven indirectly by more unspecific mechanisms, such as changes in arousal or alertness, rather than by the acoustic-electric stimulation directly. Furthermore, we observed that the connection changes from cSI and iPMC to cSII were negatively correlated to the stimulation effects on tactile perception. This suggests that the observed improvement of conscious tactile perception may be accompanied by greater excitatory projection from cSI to cSII and weaker inhibitory projection from iPMC to cSII. The alterations of these two putative connections to cSII might point to an essential role of SII in conscious tactile perception. According to previous studies, the SI and SII are responsible for transforming tactile input into tactile perception, and the SII is the first cortical region to show response differences between detected and undetected tactile stimulation (Auksztulewicz et al., 2012; Schroder, Schmidt, & Blankenburg, 2019; Wuhle, Mertiens, Ruter, Ostwald, & Braun, 2010). In combination with our modeling results, these results converge on the idea that SII is a crucial region for conscious tactile perception.

In conclusion, our study provides evidence that multimodal stimulation combining music stimulation and electrical TNS can promote conscious tactile perception in healthy human participants and suggests modulation of inter-regional cortical connections as a plausible mechanism. No such perceptual benefit could be achieved by unimodal acoustic and electric stimulation alone. In sum, this study provides insight into the reliability and functional principle of multimodal acoustic-electric stimulation, which can inspire the application of this novel approach in clinics to promote patients' consciousness.

Acknowledgements

We thank S. Kuang for the assistance in data collection. We thank Y.X. Feng for creating the head models in Fig. 1. This work was supported by Maastricht University and China Scholarship Council (CSC 201906320078 to M.W.).

Author contributions

Conceptualization: M.W., and L.R. Methodology: M.W., A.R., and L.R. Investigation: M.W. Visualization: M.W. Supervision: A.R., and L.R. Writing—original draft: M.W., and L.R. Writing—review & editing: A.R., and L.R.

Conflict of interest

All authors declare no competing interests.

Data availability

Data will be available in Open Science Framework (<https://osf.io/y9n84/>) upon publication.

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5 Supplementary materials

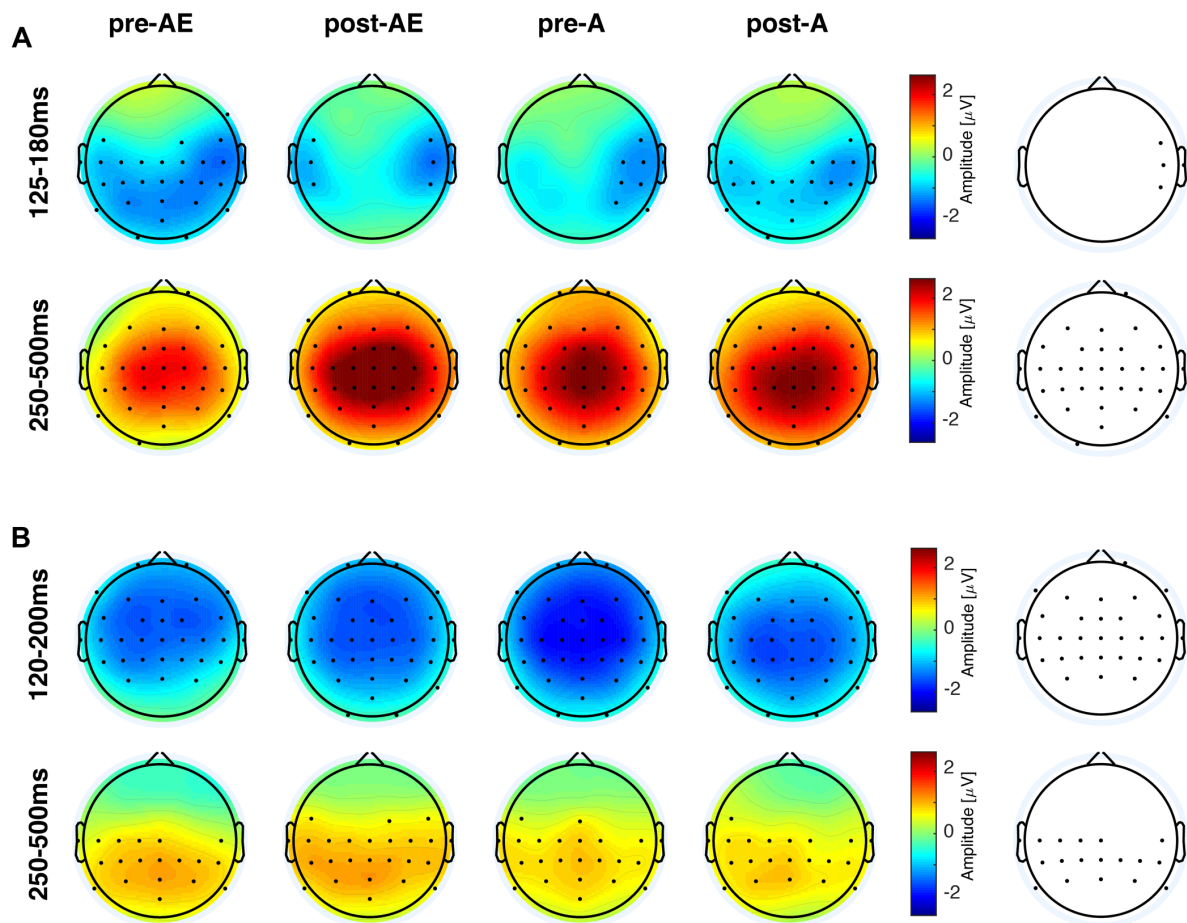


Figure S1. Topographic maps of the amplitude of awareness-related components (detected minus undetected) before and after acoustic-electric stimulation and acoustic-only stimulation. (A). The early tactile negative component was distributed over a right temporal area (upper), while the late tactile positive component was distributed over a central parietal area (bottom). **(B).** The early auditory negative component was distributed over frontal and central areas (upper), while the late auditory positive component was distributed over a parietal area (bottom). Black dots in the colored maps indicate the electrodes showing a significant difference in ERP amplitude between detected and undetected stimuli ($p < 0.05$, FDR corrected). Channels that showed such awareness-related effects reliably across all phases (pretest and posttest) and all sessions are shown in the rightmost plots.

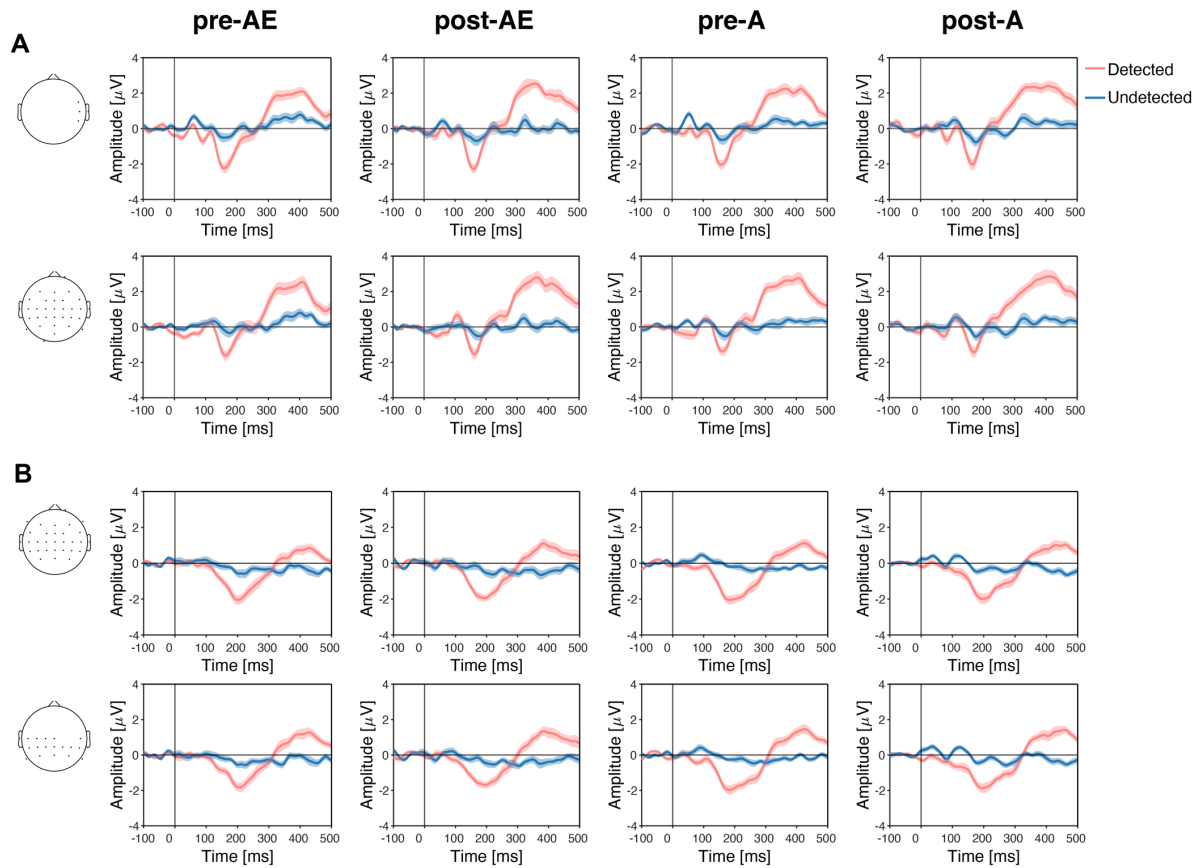


Figure S2. Neural responses to detected and undetected stimuli before and after acoustic-electric stimulation and acoustic-only stimulation. (A). Group-averaged ERP waveforms to detected (red line) and undetected (blue line) tactile stimuli averaged over a right temporal area (i.e., ROIs of the early tactile component) and a central parietal area (i.e., ROIs of the late tactile component). An early negativity and a late positivity were elicited by detected stimuli but not undetected stimuli. (B). Same as (A), but for the responses to auditory stimuli. ROIs are depicted with black dots on a head icon on the left (same as Fig.S1, right). Data are presented as mean \pm sem across participants

Chapter 4

Rhythmic musical-electrical trigeminal nerve stimulation improves impaired consciousness

Based on

Wu, M., Benyan, L., Yu, Y., Li, X., Gao, J., Li, J., Sorger, B., Riecke, L. (2022). Rhythmic Musical-Electrical Trigeminal Nerve Stimulation Improves Impaired Consciousness. *Neuroimage: Clinical*, 36, 103170.

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.

Keywords

Neural oscillations, transcutaneous stimulation, musical stimulation, disorders of consciousness, causal role

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, Massimini, Boly, & Tononi, 2016; Redinbaugh et al., 2020; Supp, Siegel, Hipp, & Engel, 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, Gorska, & Griskova-Bulanova, 2017; Cavinato et al., 2015; Gorska & 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 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 & Paulus, 2013; Jones, Johnson, Tauxe, & Rojas, 2020; Naro, Bramanti, Leo, Russo, & Calabro, 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, Khatoun, & Mc Laughlin, 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 & 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, McClure, Liebler, & Pozo-Rosich, 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, Ahonen, Evans, Freedman, & Bartel, 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 & 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 administered on five consecutive days with a single neural assessment administered on the fifth day. During the stimulation phase, stimulation was administered 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 administered 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 administered. 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.

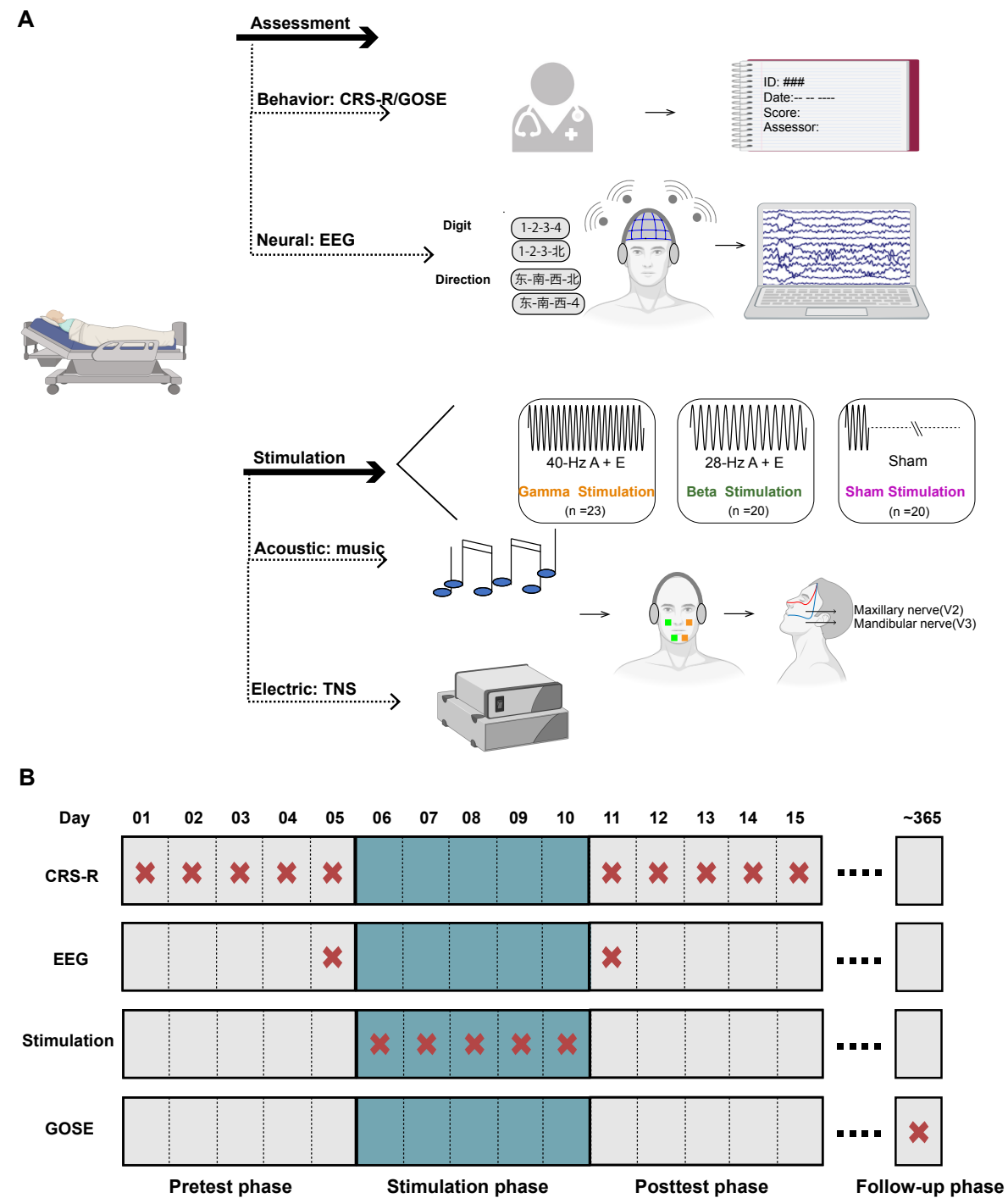


Fig. 1. Study design and experimental procedure.

(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).

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 (J. T. Giacino, Kalmar, & Whyte, 2004; J. T. 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. 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, Pettigrew, & Teasdale, 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 \pm 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 k Ω . 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, Melloni, Zhang, Tian, & Poeppel, 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 k Ω . 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 & Herrmann, 2017).

2.5 Data analysis

2.5.1 EEG data preprocessing

Data preprocessing and analysis was performed offline using EEGLAB 2019.1 (Delorme & 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 \pm 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, AbedMeraim, Cardoso, & Moulines, 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: $15.5\% \pm 9.1\%$; mean \pm SD across patients) using the EEGLAB plugin ICLables (Pion-Tonachini, Kreutz-Delgado, & Makeig, 2019). The weights of the non-artifactual components were reapplied to the original unfiltered channel data (Jaeger, Bleichner, Bauer, Mirkovic, & Debener, 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 (Kallionpää 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, Cherkin, Sherman, Goldberg, & Pressman, 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.

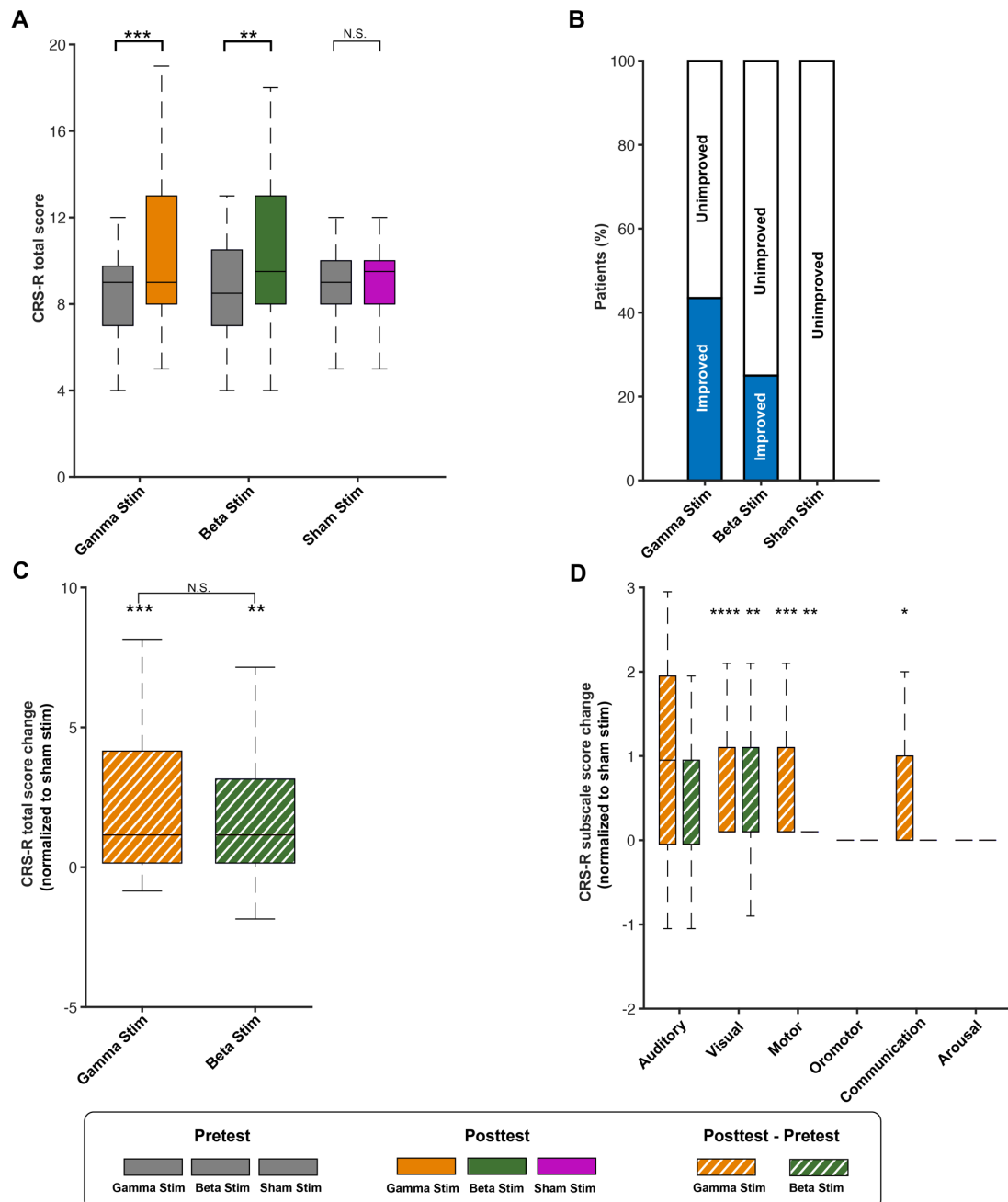


Fig. 2. Consciousness level in DOC patients before and after rhythmic musical-electrical TNS or sham stimulation.

(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, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

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 of similar strength in the three groups (no effect of *stimulation type*: gamma ASSR: $F_{2,59} = 0.324$, $p = 0.725$, effect size $\eta^2_p = 0.011$; 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]).

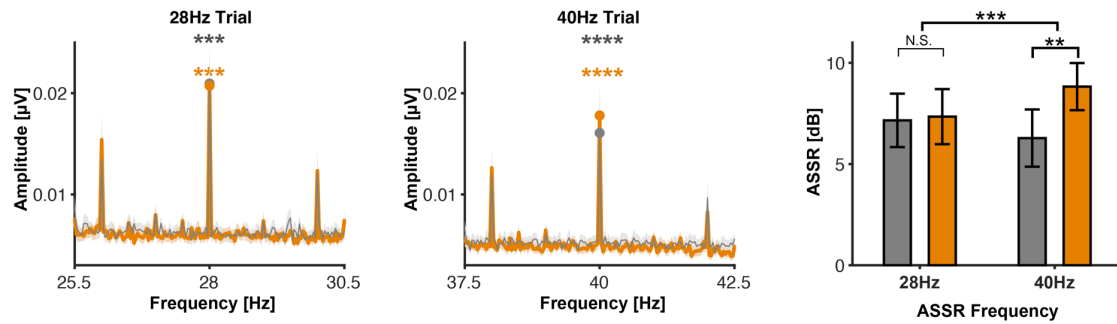
Importantly, comparison of these rhythmic cortical responses after vs before stimulation revealed a significant positive effect of gamma stimulation on gamma responses ($t_{21} = 3.056$, $p = 0.006$, effect size $d = 0.652$; Fig. 3A, right) but not beta responses ($t_{21} = 0.262$, $p = 0.796$, $d = 0.056$), with a significantly stronger effect on gamma responses than beta responses (interaction *time* [pretest, posttest] \times ASSR *frequency* [beta, gamma]: $F_{1,21} = 15.557$, $p = 7.4 \times 10^{-4}$, $\eta^2_p = 0.426$). 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* \times 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-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* \times ASSR *frequency*: $F_{1,18} = 0.091$, $p = 0.767$, $\eta^2_p = 0.005$, Fig. 3C, right). The

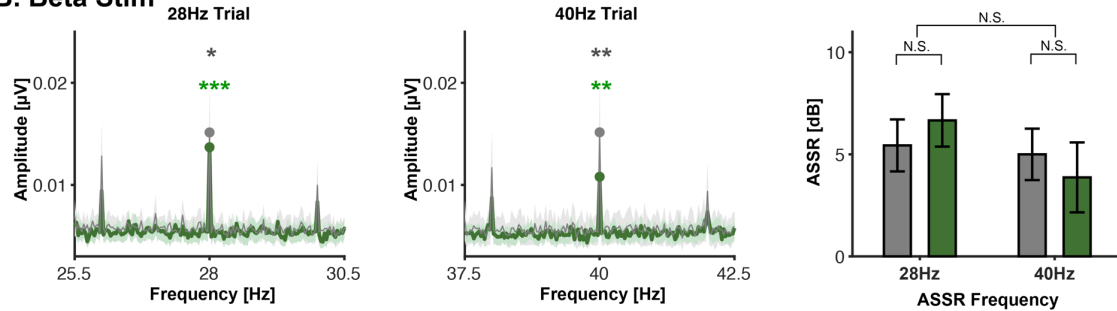
non-significant effect of sham stimulation on gamma responses was significantly smaller than the effect of gamma, but not beta, stimulation (interaction *time* × *stimulation type* [gamma stimulation, sham stimulation]: $F_{1,39} = 8.077$, $p = 0.004$, $\eta^2_p = 0.172$; interaction *time* × *stimulation type* [beta stimulation, sham stimulation]: $F_{1,36} = 0.040$, $p = 0.421$, $\eta^2_p = 0.001$). 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]: $F_{1,36} = 3.515$, $p = 0.024$, $\eta^2_p = 0.089$; interaction *time* × *stimulation type* [gamma stimulation, sham stimulation]: $F_{1,39} = 2.136$, $p = 0.076$, $\eta^2_p = 0.052$).

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.

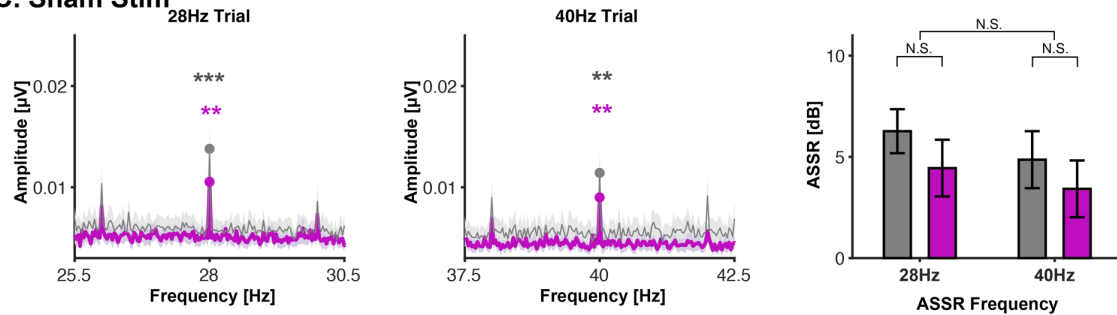
A. Gamma Stim



B. Beta Stim



C. Sham Stim



D

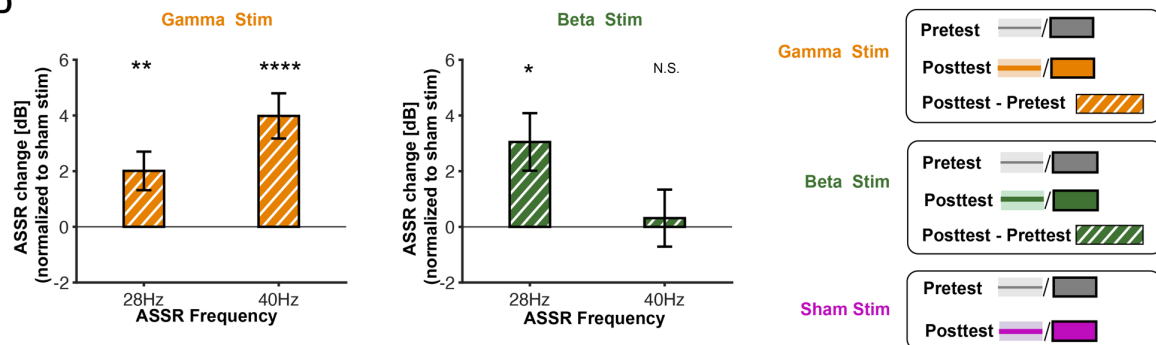


Fig. 3. Rhythmic brain responses to amplitude-modulated speech in DOC patients before and after rhythmic musical-electrical TNS or sham stimulation.

(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 \pm sem across participants. n.s. non-significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

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.

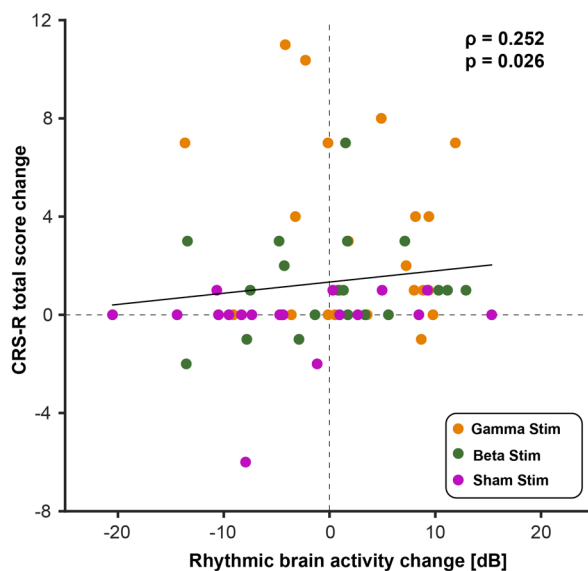


Fig. 4. Relation between changes in consciousness and changes in rhythmic brain activity in DOC patients. The scatterplot shows results from a correlation analysis testing for a functional coupling between changes in consciousness and changes in rhythmic brain activity. Correlation coefficient rho (ρ) 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.

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 Figs. 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.

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	-.041	.960	0.912	1.010	.112			
Etiology/TBI	.047	1.048	0.214	5.129	.953			
Month since injury	.234	1.264	0.970	1.644	.083	.572	27.347	4.9×10^{-5}
CRS-R total score	.573	1.774	1.245	2.529	.002			
Response to TNS/Yes	2.484	11.989	2.092	68.717	.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, Bruno, Ledoux, Demertzi, & Laureys, 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 (Joseph T. Giacino, Fins, Laureys, & Schiff, 2014; J. T. 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, Owen, & Schiff, 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, Bramanti, Leo, Bramanti, & Calabro, 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, Dowsett, & Herrmann, 2016) and the duration of such aftereffects seems to be positively related to the duration of the stimulation (Nitsche et al., 2003; Vossen, Gross, & Thut, 2015). Moreover, the strength of these aftereffects may be positively related to the strength of the instantaneous effects of the stimulation (Helfrich, Knepper, et al., 2014; Helfrich, Schneider, et al., 2014). 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 adaptations in temporal patterns of synaptic activity (Feldman, 2012; Zaehle, Rach, & Herrmann, 2010). These adaptations may persist and reverberate until after the stimulation, resulting in a long-lived rhythmic aftereffect (Caporale & 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, Lythe, & Green, 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 & Poeppel, 2012; Preisig et al., 2020). Moreover, detection of changes in repeated utterances has been shown to lead to increased gamma activity (Basirat, Sato, Schwartz, Kahane, & Lachaux, 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, Lopez, & Cornejo, 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, Perrin, Campbell, Danish, & Goldstein, 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 & Butt, 2001; Edlow, Claassen, Schiff, & Greer, 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.

4.7 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.

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

Data availability statement

Data will be deposited in Open Science Framework (<https://osf.io/d7xaw/>) upon publication.

Ethical approval and consent to participate

The study was approved by the ethics committee of Hangzhou Mingzhou Brain Rehabilitation Hospital (2020003) and was carried out in accordance with the Declaration of Helsinki. Informed consent of all enrolled patients was obtained from the patients' legal surrogates prior to the study.

Funding

This work was supported by the National Natural Science Foundation of China (81870817) to B.L. and China Scholarship Council to M.W. (CSC 201906320078).

Competing interests

Authors declare no competing interests relevant to this work.

Author contributions

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.

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5 Supplementary materials

This file includes:

Main Text (Results)

Figures S1 to S4

Tables S1 to S2

Results

Neural tracking of hierarchical linguistic structures

To assess neural tracking of hierarchical linguistic structures, we analyzed the ASSR at the sentence rate (0.5 Hz) and word rate (2 Hz). Patients in all three stimulation groups showed clear spectral peaks at the word rate, indicating significant neural tracking of words (spectral peak > noise floor, one-tailed paired t-test, FDR-corrected; Fig. S2A-C, left). A subset of patients also showed spectral peaks at the sentence rate and its second harmonic (1 Hz); however, these peaks were not observed reliably across groups or sessions, probably reflecting the fact that the patients had limited consciousness (Gui et al., 2020). Overall, we found musical-electrical TNS to have no systematic effect on the neural tracking of words or sentences. We observed a reduction in word tracking after beta stimulation ($t_{18} = 2.239$, $p = 0.038$, $d = 0.514$; Fig. S2B right); however, this change was not significantly larger than that observed after sham stimulation ($t_{18} = 2.048$, $p = 0.055$, $d = 0.470$; Fig. S2D right). Beta stimulation improved sentence tracking (beyond sham stimulation-related changes, $t_{18} = 3.254$, $p = 0.004$, $d = 0.747$; Fig. S2D right); however, this change did not render sentence tracking significant in these patients ($t_{18} = 1.523$, $p = 0.145$, $d = 0.349$; Fig. S2B). In sum, these supplementary results suggest that the rhythmic musical-electrical TNS did not systematically facilitate DOC patients' neural tracking of hierarchical linguistic structures.

Neural detection of semantic violations

To assess neural detection of semantic violations, we computed ERPs evoked by semantically congruent words vs. semantically incongruent words. This paradigm has been shown to elicit a negative ERP component around 400 ms (relative to the onset of the word) that is commonly thought to encode semantic violations (Kutas & Hillyard, 1980; Rohaut et al., 2015). Overall, we did not find this ERP component reliably across patients or sessions, probably due to the fact that patients had limited consciousness (Fig. S3A-C, left and center). However, we observed a visibly more negative response to incongruent vs congruent words at around 300-500 ms in the gamma-stimulation group in the posttest (Fig. S3A center), suggesting that these patients could detect semantic violations after they underwent gamma stimulation.

To test whether musical-electrical TNS improved semantic violation detection we statistically compared the difference waveforms (incongruent minus congruent) in posttest vs pretest with cluster-based permutation tests. This revealed a significantly enlarged negative difference from 227 to 396 ms after word onset in the gamma stimulation group (Fig. S3A, right), supporting our notion above that gamma stimulation improved semantic violation detection. We did not observe such an improvement in the beta or sham stimulation group (Fig. S3B-C, right). Analysis of the unbiased neural effect of gamma stimulation (corrected for the observed average sham-stimulation-related change) further corroborated our observation above, revealing a significant increase in semantic violation detection in posttest vs pretest from 223 to 408 ms (Fig. S3D, left). No such significant change was detected at any latency in the beta stimulation group (Fig. S3D, right). These results indicate that

rhythmic musical-electrical TNS at gamma, but not beta, frequency may improve neural detection of semantic violations in DOC patients (beyond sham stimulation-related changes).

Susceptibility of MCS and UWS patients' consciousness to rhythmic stimulation

To identify DOC patients who benefitted particularly strongly from the rhythmic musical-electrical TNS, we explored the sizes of the observed behavioral and neural stimulation effects in DOC patients with different diagnoses at pretest. Fig. S4 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. S4A-B and S4D-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 S1, Fig. S4A, S4D, 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-electrical TNS, with a stronger benefit for MCS patients. Fig. S4C and S4F show the neural benefit for each diagnostic group. Neural benefits also showed a similar pattern within each group, suggesting that rhythmic musical-electrical 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.

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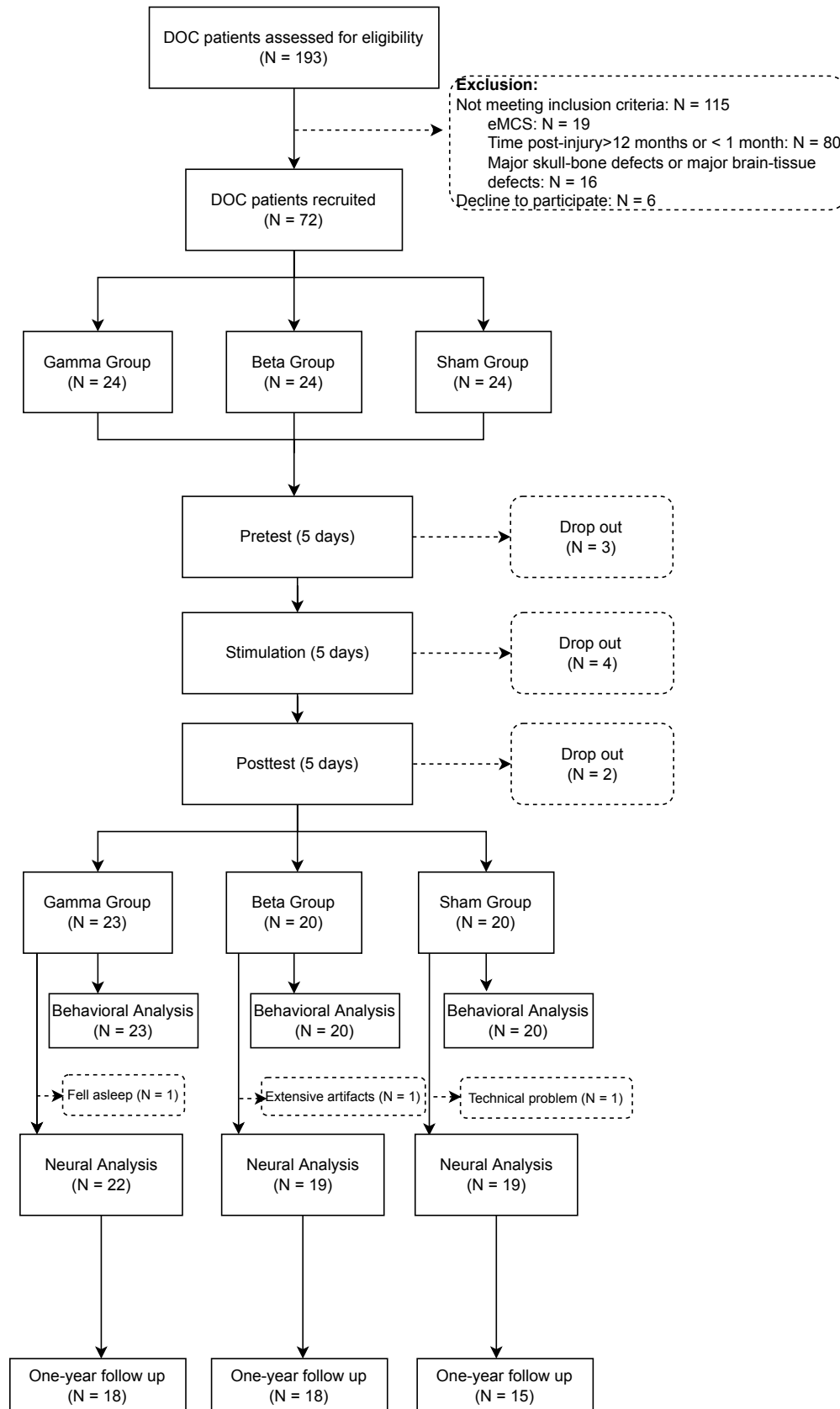


Fig. S1. Flow chart of the procedures.

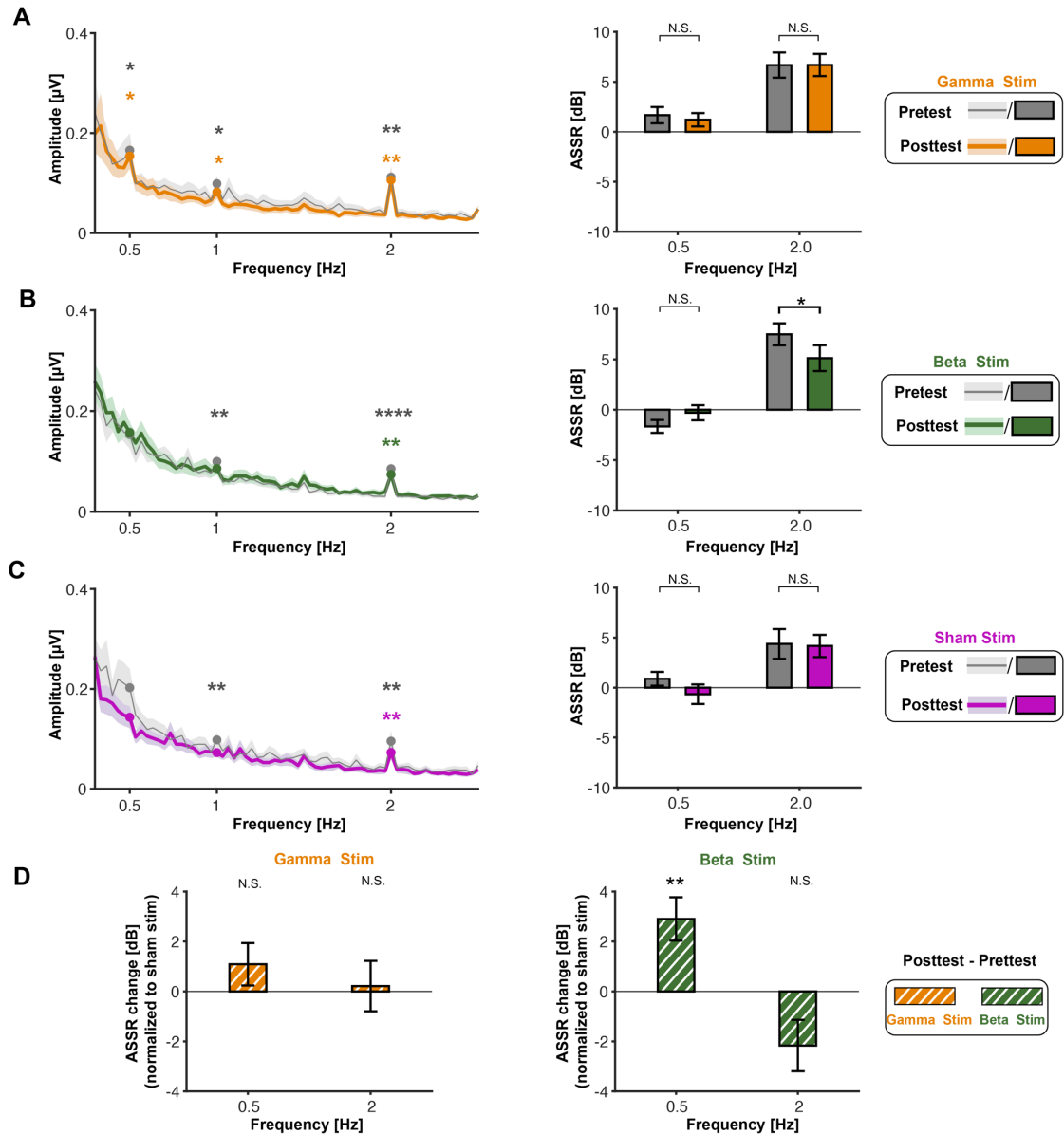


Fig. S2. Neural tracking of hierarchical linguistic structures in DOC patients before and after rhythmic musical-electrical TNS or sham stimulation.

(A). Spectral response to hierarchical linguistic structures in pretest (gray) and posttest (orange) for the gamma-stimulation group. The left plot reveals spectral peaks at the word rate (2 Hz) and to a lesser degree at sentence rate (0.5 Hz) and its second harmonic (1 Hz). The bar plot (right) shows the ASSR at word rate and sentence rate in pretest (gray) and posttest (orange).

(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 ASSR at sentence rate and word rate, after correcting for sham stimulation-related changes (see Methods).

Data are presented as mean \pm sem across participants. n.s. non-significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

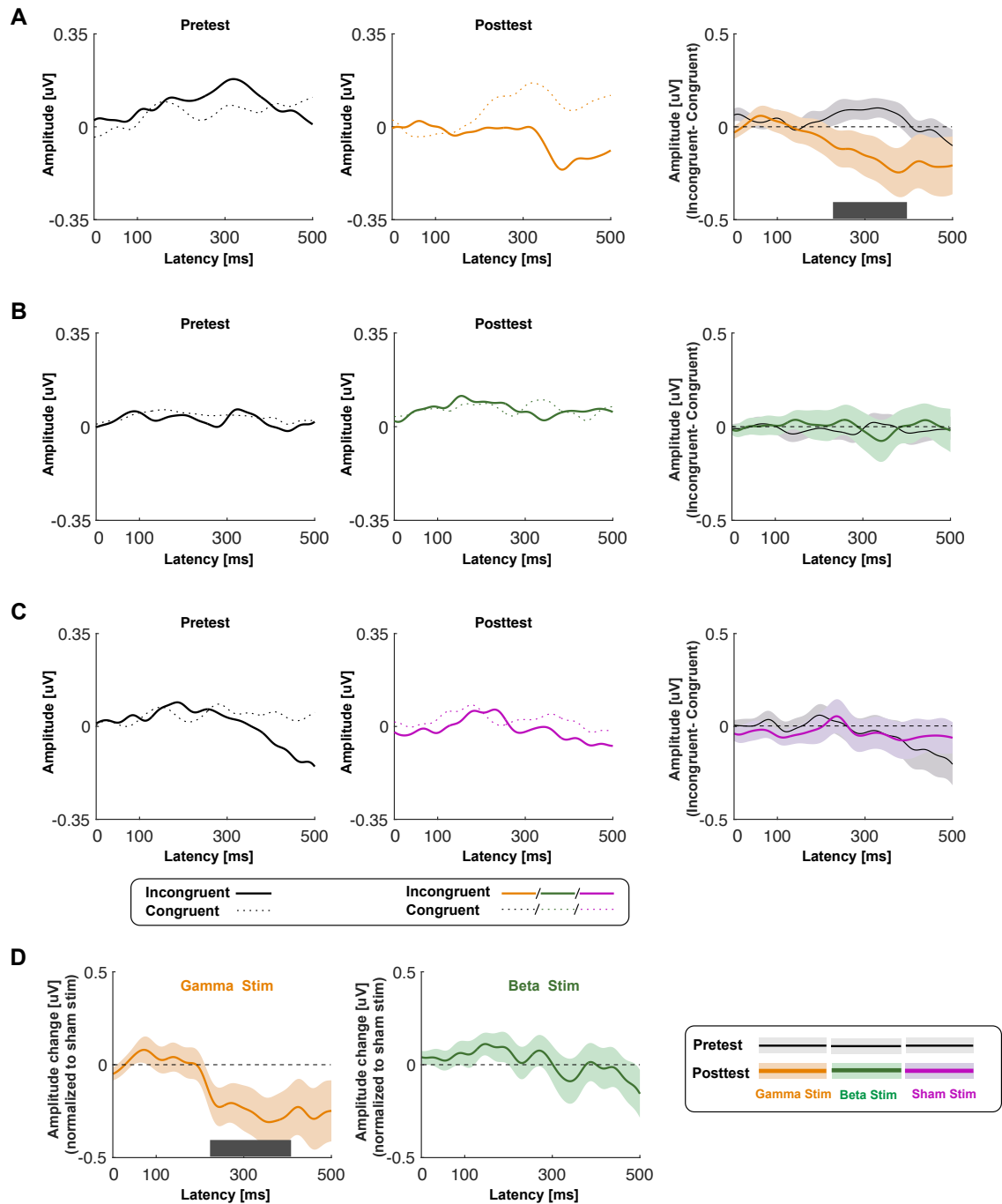


Fig. S3. Neural detection of semantic violations in DOC patients before and after rhythmic musical-electrical TNS or sham stimulation.

(A). Grand-average ERP waveforms to the same word presented in semantically congruent context (dashed line) or semantically incongruent context (solid line) in pretest (left) and posttest (center) for the gamma stimulation group. Zero latency represents the onset of the word. Semantically

incongruent words evoked more negative ERPs at ~300-500 ms than acoustically-matched semantically congruent words in posttest. The right plot shows difference ERP waveforms (incongruent minus congruent) representing the time course of semantic violation detection in pretest (gray) and posttest (orange). Gamma stimulation had a positive aftereffect on putative semantic violation detection throughout a time window between 227-396 ms.

(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) and beta stimulation (right) on the time course of semantic violation detection, after correcting for sham stimulation-related changes (see Methods). Negative values indicate intervals during which the musical-electrical TNS improved semantic violation detection beyond sham stimulation-related changes.

Data are presented as mean \pm sem (shaded area in panels A-C, right and panel D\)) across participants. The black horizontal bars represent clusters of time points showing a statistically significant difference (permutation test, corrected $p < 0.05$).

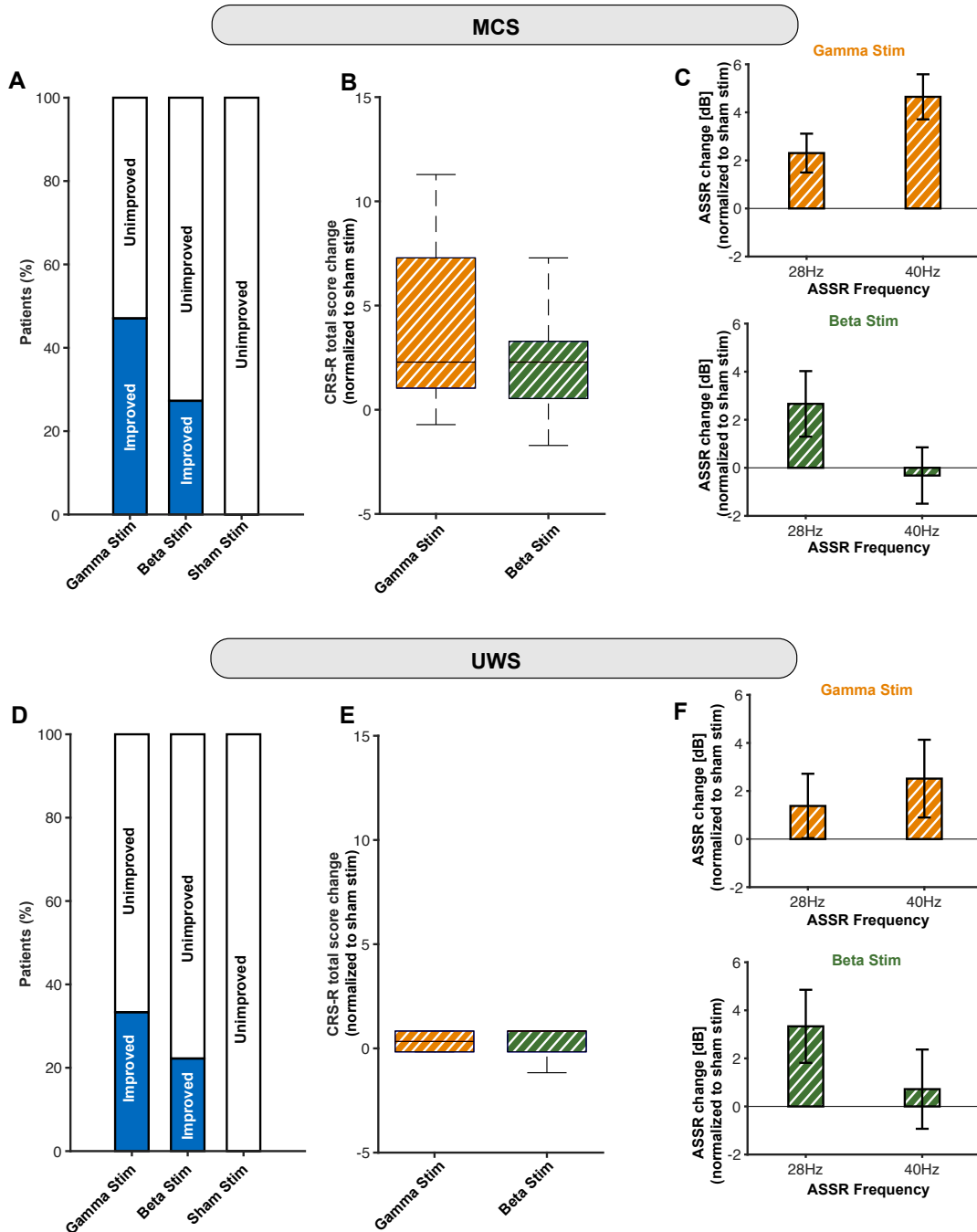


Fig. S4. Changes in consciousness level and rhythmic brain activity induced by rhythmic musical-electrical TNS in MCS and UWS patients.

(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 and B; N = 16 in panel C; beta-stimulation group: N = 11; sham-stimulation group: N = 13 in panels A and 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 and 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 \pm sem across participants.

Table S1. Demographic and clinical characteristics of the DOC patients.

Stimulation	Patient number	Age (years)	Gender	Etiology	Time since injury (months)	Pretest			Posttest			Improvement of diagnosis	GOSE score
						CRS-R subscores	CRS-R total score	Diagnosis	CRS-R subscores	CRS-R total score	Diagnosis		
Gamma	1	70	M	CVA	4.0	011102	5	UWS	231102	9	MCS-	Y	2
	2	38	F	CVA	3.0	132102	9	MCS-	343012	13	MCS+	Y	3
	3	68	M	TBI	8.5	112102	7	UWS	112102	7	UWS	N	1
	4	48	M	CVA	2.0	031102	7	MCS-	032102	8	MCS-	N	1
	5	44	F	CVA	2.0	112102	7	UWS	122102	8	MCS-	Y	2
	6	33	M	CVA	3.5	132102	9	MCS-	334102	13	MCS+	Y	3
	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	3
	9	62	F	ABI	5.5	111100	4	UWS	112100	5	UWS	N	2
	10	42	M	ABI	5.5	232102	10	MCS-	453123	18	EMCS	Y	4
	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	1
	13	37	M	CVA	7.0	032002	7	MCS-	032102	8	MCS-	N	3
	14	41	M	CVA	3.5	131102	8	MCS-	232102	10	MCS-	N	3
	15	63	F	CVA	1.5	222102	9	MCS-	132102	9	MCS-	N	2
	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	4
	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	1
	21	43	F	TBI	3.5	232102	10	MCS-	332212	13	MCS+	Y	2
	22	70	F	TBI	7.0	222102	9	MCS-	222102	9	MCS-	N	2
	23	72	F	TBI	8.5	232102	10	MCS-	233102	11	MCS-	N	2
Beta	24	40	F	ABI	9.0	352102	13	MCS+	354102	15	MCS+	N	3
	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	3
	28	70	M	CVA	5.0	114102	9	MCS-	124102	10	MCS-	N	2
	29	41	M	TBI	6.5	112002	6	UWS	322112	11	MCS+	Y	4
	30	79	M	TBI	3.5	211102	7	UWS	212102	8	UWS	N	2
	31	47	M	TBI	1.5	235102	13	MCS-	345112	16	MCS+	Y	5
	32	67	M	ABI	1.5	215102	11	MCS-	225102	12	MCS-	N	3
	33	79	M	TBI	6.0	115102	10	MCS-	334102	13	MCS+	Y	3
	34	56	F	TBI	6.5	212102	8	UWS	212102	8	UWS	N	2
	35	39	M	TBI	1.5	112102	7	UWS	212102	8	UWS	N	2

	36	31	M	CVA	1.0	212102	8	UWS	212102	8	UWS	N	2
	37	59	M	CVA	1.0	232102	10	MCS-	235102	13	MCS-	N	3
	38	44	F	CVA	2.0	334102	13	MCS+	354112	16	MCS+	N	3
	39	79	F	ABI	2.0	112102	7	UWS	212102	8	UWS	N	2
	40	32	M	CVA	2.0	102100	4	UWS	102100	4	UWS	N	1
	41	61	M	CVA	1.0	212100	6	UWS	112100	5	UWS	N	1
	42	50	M	ABI	1.0	212102	8	UWS	222102	9	MCS-	Y	2
	43	37	M	CVA	1.0	232102	10	MCS-	232102	10	MCS-	N	2
	44	46	F	ABI	3.0	101102	5	UWS	101102	5	UWS	N	2
	45	54	M	CVA	4.0	132102	9	MCS-	232102	10	MCS-	N	2
	46	49	M	CVA	4.0	132102	9	MCS	112102	7	UWS	N	1
	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	1
	49	56	F	TBI	5.0	222102	9	MCS-	232102	10	MCS-	N	2
	50	70	M	TBI	8.5	355102	16	MCS+	232102	10	MCS-	N	2
	51	66	M	TBI	1.0	212102	8	UWS	212102	8	UWS	N	2
	52	60	F	CVA	1.0	232102	10	MCS-	232102	10	MCS-	N	3
Sham	53	57	F	ABI	3.0	212102	8	UWS	212102	8	UWS	N	2
	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	1
	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	3
	59	44	F	CVA	4.0	222102	9	MCS-	222102	9	MCS-	N	2
	60	70	M	CVA	6.0	335112	15	MCS+	335112	15	MCS+	N	2
	61	37	M	CVA	9.0	232102	10	MCS-	232102	10	MCS-	N	3
	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	1

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 13th column highlights patients whose best diagnosis improved from pretest to posttest phase. The final column depicts GOSE score in the one-year follow-up phase (slashes indicate patients who dropped out from the study after 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; GOSE, Glasgow Outcome Scale-Extended

Table S2. Group analysis of demographic and clinical characteristics of the DOC patients in three groups

Variable		Gamma stim (N =23)	Beta stim (N=20)	Sham stim (N=20)	Test statistic	p-Value
Age (year)	Mean \pm SD	51 \pm 14	54 \pm 16	54 \pm 13	$F_{(2,62)} = 0.391$	0.678
	Min-Max	24-72	31-79	24-74		
Gender	Female	10	5	8	$\chi^2 = 1.703$	0.427
	Male	13	15	12		
Etiology	TBI	6	7	6	$\chi^2 = 0.397$	0.820
	Non-TBI	17	13	14		
Time since injury (month)	Mean \pm SD	4.4 \pm 2.6	3.8 \pm 3.3	5.1 \pm 3.3	$F_{(2,62)} = 0.951$	0.392
	Min-Max	1-10	1-11	1-11		
CRS-R in pretest	Median	9.0	8.5	9.0	$\chi^2 = 1.723$	0.422
	Min-Max	4-12	4-13	5-16		
Diagnosis	MCS	17	11	14	$\chi^2 = 1.839$	0.399
	UWS	6	9	6		

One-way ANOVAs: Age, time since injury; Kruskal-Wallis H test: gender, etiology, CRS-R in pretest, diagnosis

Chapter 5

Acoustic-electric trigeminal-nerve stimulation enhances functional connectivity in patients with disorders of consciousness

Based on

Wu, M., Concolato, M., Sorger, B., Yu, Y., Li, X., Luo, B., Riecke, L. (2023). Acoustic-electric trigeminal-nerve stimulation enhances functional connectivity in patients with disorders of consciousness, *CNS Neuroscience & Therapeutics*, in press

Abstract

Disruption of functional brain connectivity is thought to underlie disorders of consciousness (DOC). Recovery of impaired connectivity has been suggested as an indicator of consciousness restoration. We recently found that rhythmic acoustic-electric trigeminal-nerve stimulation (i.e., musical stimulation synchronized to transcutaneous oscillatory electrical stimulation of the trigeminal nerve) in the gamma band can improve consciousness in patients with DOC. However, whether these beneficial stimulation effects are mediated by alterations in functional connectivity has not been investigated so far. In this study, we analyzed neural and behavioral data of 63 patients with DOC who underwent five days of gamma, beta, or sham acoustic-electric trigeminal-nerve stimulation. Resting-state electroencephalography was measured before and after the stimulation and resting-state functional connectivity was assessed using phase-lag index (PLI). We found that gamma stimulation induces an increase of PLI in the gamma band. Further characterization revealed that the enhancing effect is (i) specific to the gamma band (as we observed no comparable change in beta-band PLI and no effect of beta-band acoustic-electric stimulation or sham stimulation), (ii) widely spread across the cortex, and (iii) accompanied by improvements in patients' auditory abilities. These findings thus show that gamma acoustic-electric trigeminal-nerve stimulation can improve resting-state functional connectivity in the gamma band, which in turn may be linked to auditory abilities and/or consciousness restoration in DOC patients.

Keywords

Resting-state functional connectivity, gamma band, electric trigeminal-nerve stimulation, acoustic stimulation, disorders of consciousness

1 Introduction

Even though the mechanisms underlying disorders of consciousness (DOC) remain poorly understood, a growing body of evidence is accumulating regarding the neural correlates of DOC (Bai, Xia, & Li, 2017; Di Perri, Stender, Laureys, & Gosseries, 2014). Some neuroimaging studies have demonstrated abnormalities in the structural and functional connectivity of DOC patients' brains (Tan et al., 2019; Vanhaudenhuyse et al., 2010). It has been suggested that a functional disconnection, especially of long-range corticocortical and thalamocortical connections, is a candidate mechanism underlying impairments of consciousness in DOC patients, and that recovery of consciousness is accompanied by partial restoration of these connections (Bodien, Chatelle, & Edlow, 2017; Laureys et al., 2000). These assumptions are further supported by several resting-state electroencephalographic (EEG) studies showing alterations in functional connectivity (quantified as synchronization of oscillations between different areas) in various oscillatory frequency bands in DOC patients (Chennu et al., 2017; Chennu et al., 2014; Lehembre et al., 2012; Schorr, Schlee, Arndt, & Bender, 2016). Although the results of these studies are heterogeneous, some suggest an association between increased functional connectivity and increased level of consciousness or improved DOC-treatment outcome (Chennu et al., 2017; Lehembre et al., 2012; Naro, Calabrò, et al., 2018).

Thalamocortical gamma oscillations, particularly at 40 Hz, have been proposed as a substrate for consciousness (Llinas, Ribary, Contreras, & Pedroarena, 1998; Negrao & Viljoen, 2009). For example, brain activity in the low gamma range (around 40 Hz) in response to rhythmic auditory stimuli has been suggested as a marker of the integrity of thalamocortical networks in prolonged DOC patients (Binder, Gorska, Pipinis, Voicikas, & Griskova-Bulanova, 2020). In fact, activity in this range has been shown to correlate positively with consciousness level in prolonged-DOC patients (Binder, Gorska, & Griskova-Bulanova, 2017; Gorska & Binder, 2019) and lucid dreaming in healthy subjects (Voss et al., 2014; Voss, Holzmann, Tuin, & Hobson, 2009). Furthermore, functional connectivity in the high gamma range has been associated with accelerated recovery of consciousness after anesthesia in rats (Li, Hambrecht-Wiedbusch, & Mashour, 2017) and with changes in sleep vs. wakefulness state in human subjects (Mikulan et al., 2018). Thus, targeting oscillations and functional connectivity in the gamma band might be a potential therapeutic avenue for DOC.

A common approach to modulate neural oscillations in a specific band involves the non-invasive application of rhythmic sensory or electrical stimulation. We have recently proposed and validated a novel variant involving the combined application of rhythmic musical stimulation and rhythmic transcutaneous electrical stimulation of the trigeminal nerve (TN) (referred to as "acoustic-electric TN stimulation") (Wu et al., 2022). In that study, we found that acoustic-electric TN stimulation in beta (28 Hz) and especially gamma (40 Hz) band has beneficial effects on DOC patients' level of consciousness and neural oscillatory responses at the stimulation frequency. In particular, 40 min of stimulation per day, for five consecutive days, lead to long-lasting improvements in both the level of consciousness and steady-state responses to auditory stimulation (ASSRs) at the stimulation frequency.

In fact, each type of gamma stimulation – rhythmic acoustic stimulation and oscillatory electrical stimulation – has previously been shown to entrain gamma oscillations in animals and healthy human individuals (Martorell et al., 2019; Voss et al., 2014) and to modulate resting-state functional connectivity in DOC patients (Naro, Bramanti, Leo, Russo, & Calabro, 2016). Additionally, 40-Hz trigeminal-nerve stimulation has been reported to improve consciousness in a DOC patient and this improvement was accompanied by changes in functional connectivity, as measured with functional magnetic resonance imaging (fMRI) (Fan et al., 2019). According to the current body of evidence, we hypothesized that (i) gamma acoustic-electric TN stimulation can improve gamma-band functional connectivity in DOC patients and (ii) the putative change in resting-state functional connectivity is the mechanism underlying the beneficial effects of TN stimulation on consciousness level and neural oscillatory responses as found in our previous study.

2 Methods

2.1 Patients

Data were analyzed from 63 patients diagnosed with DOC based on Coma Recovery Scale-Revised (CRS-R) (21 in an unresponsive wakefulness syndrome [UWS], 42 in a minimally conscious state [MCS]), which were obtained in our previous study (Wu et al., 2022). Initially, a total of 72 patients were recruited and assigned to three groups matched as much as possible for demographic characteristics (i.e., age, etiology and time since injury) and CRS-R score at pretest. Sixty-three out of 72 patients completed the study and were included for the current analysis, resulting in 23 patients in the gamma-TN stimulation group, 20 patients in the beta group and 20 patients in the sham group. Detailed demographic data of the patients and matching procedures are available in (Wu et al., 2022) (supplementary Table S1 and Methods-Patients). The study was approved by the local research-ethics committee and registered as a clinical trial on www.clinicaltrials.gov (NCT04435301). Informed consent was obtained from the patients' legal surrogates.

2.2 Study design

The study followed a mixed 2×3 design with the within-subject factor *time* (pretest, posttest) and the between-subject factor *stimulation* (gamma, beta, sham). The study consisted of three consecutive phases of five days each: pretest phase, stimulation phase, and posttest phase (Fig. 1). Behavioral assessments were administrated during pretest and posttest phases on each of five consecutive days, as repeated CRS-R assessments have been shown to improve diagnostic accuracy (Wannez et al., 2017). A single session of neural assessment (ca. 55 min of continuous EEG recording) was performed on the fifth day during the pretest phase and on the first day of the posttest phase. During the stimulation phase, each group of patients received a single session of gamma, beta or sham acoustic-electric TN stimulation on each of the five consecutive days. The beta and sham stimulation were included to further test whether putative stimulation effects on connectivity were specific to the gamma stimulation frequency and caused by the actual acoustic-electric TN stimulation (but not a placebo effect).

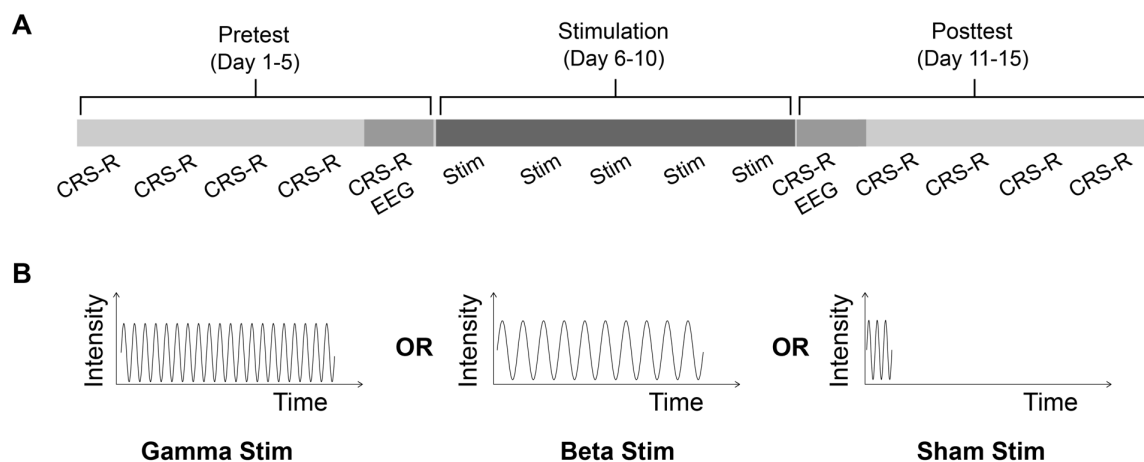


Figure 1. Schematic of experimental procedure. (A) Each patient underwent a 15-day long experimental procedure consisting of three 5-day long consecutive phases: pretest, stimulation, and posttest. The pretest and posttest phases involved administration of daily behavioral assessments

(CRS-R) and a single neural assessment (ca. 55 min EEG) on the day immediately before and after the stimulation phase, respectively. (B) The stimulation phase involved daily administration of 40 min of rhythmic gamma (left) or beta (center) acoustic-electric TN stimulation, or sham (right) stimulation.

2.3 Stimulation

The stimulation phase involved administration of rhythmic acoustic-electric stimulation, composed of simultaneous musical stimulation and trigeminal nerve stimulation.

Acoustic stimulation was a continuous 40 min long musical stream (ten concatenated pieces of music). Amplitude modulations (AM) with 40 Hz or 28 Hz (gamma stimulation and beta stimulation, respectively; sinusoidal waveform, modulation depth: 100%) were applied to the sequence. The onset/offset of each excerpt was ramped up/down using 5 s long ramps. The acoustic stimulation was presented diotically through insert earphones at a fixed sound level (50 dB SPL).

Electric stimulation consisted of non-invasive application of alternating currents to the patients' face to facilitate rhythmic TN activity. The aforementioned AM and ramping up/down steps were applied analogously to the current waveform. The current intensity was fixed at ± 8 mA. The current was applied using two pairs of square-shaped rubber electrodes (size: 3 cm \times 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 (maxillary nerve and mandibular nerve, respectively). The electrodes were adhered to the patients' skin using conductive paste and the impedance was kept below 5 k Ω . The electric stimulation was delivered simultaneously with the acoustic stimulation using a battery-powered DC stimulator (DCSTIMULATOR MC, NeuroConn, Germany) and lasted 40 min.

Sham stimulation was identical to the gamma stimulation above, except that the acoustic and electric stimulation were slowly ramped down after the first 30 s of stimulation. Note that 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. More detailed description of acoustic-electric TN stimulation can be found in (Wu et al., 2022).

2.4 Behavioral assessment

Patients' level of consciousness was assessed by two blinded clinicians using the CRS-R during pretest and posttest phases (Giacino, Kalmar, & Whyte, 2004).

2.5 Neural assessment

2.5.1 EEG recording

EEG data were collected at the patients' bedside using a 64-channel active BrainCap (Brain Products GmbH, Gilching, Germany) in the standard 10-20 system. An additional electrooculography electrode was placed at the suborbital ridge of the right eye. The ground electrode was AFz and all EEG electrodes were referenced to scalp position FCz. Electrode impedances were kept below 10 k Ω . The EEG recordings were online bandpass-filtered between 0.01 Hz and 70 Hz, and digitized with a sampling rate of 1 kHz. The EEG recording included two sessions, respectively at pretest and at posttest, each including 5 min of resting state and approximately 50 min of passive listening to amplitude-modulated speech. The speech stimuli were repetitive word quartets. Half of the stimuli carried the 40-Hz AM and the other half carried the 28-Hz AM. The AM speech served the assessment of the ASSR (Wu et al., 2022).

2.5.2 EEG preprocessing

Data preprocessing and analysis were performed offline using EEGLAB 2019.1 (Delorme & Makeig, 2004) and MATLAB 9.4. First, bad channels were identified based on voltage distribution, with kurtosis higher than five as a criterion for rejection. These channels were replaced by interpolating between the surrounding electrodes (spherical spline interpolation; number of interpolated channels: 2.4 ± 2.2 , mean \pm SD across patients). Second, data were band-pass filtered between 1 Hz and 45 Hz using a Butterworth Infinite Impulse Response (IIR) filter (zero phase shift, filter order: 6). Third, the interpolated channel data were re-referenced to an average reference. Fourth, independent component analysis was applied to the data using a second-order blind-identification algorithm (Belouchrani, AbedMeraim, Cardoso, & Moulines, 1997). Artifactual components were identified and discarded (number of artifactual components: 21.7 ± 8.1 ; mean \pm SD across patients) using the EEGLAB plugin ICLables (Pion-Tonachini, Kreutz-Delgado, & Makeig, 2019). The data were reconstructed based on the remaining components. Next, the artifact-reduced, continuous data were separately filtered into beta (12-30 Hz) and gamma (30-45 Hz) bands using the same IIR filter as above. Finally, the filtered data were segmented into 4-s non-overlapping epochs, resulting in 75 epochs per patient that were used for further analysis.

2.5.3 Functional connectivity

Functional connectivity was assessed using a phase-lag index (PLI) that quantifies phase synchronization between two time-varying signals and is considered to be less sensitive to the undesired influence of common sources (volume conduction and/or active reference electrodes) compared to other commonly used measures of connectivity (Stam, Nolte, & Daffertshofer, 2007). The PLI quantifies the degree to which two signals are consistently either in phase advance (phase lead) or in phase delay (phase lag) with each other, and disregards phase lags of 0 modulo π , that are attributed to the influence of common sources. PLI values range from 0 to 1, with 0 representing no phase coupling, and 1 representing perfect phase coupling; the latter can be interpreted as one signal being consistently either in phase advance or in phase delay with respect to the other. The calculation of PLI followed the steps below and was conducted separately for the gamma and beta band: first, within each epoch, the instantaneous phase was determined using the Hilbert transformation. Then, the PLI between two signals for a certain epoch was obtained from a time series of phase differences $\Delta\phi(t_k)$, $k = 1 \dots N$ according to the formula $PLI = |\langle \text{sign}[\sin\Delta\phi(t_k)] \rangle|$, where $\Delta\phi(t_k)$ indicates the difference between the instantaneous phases of two channels at time point t_k . Finally, the mean PLI for each pair of channels was calculated as the arithmetic mean of all epochs and then represented as a 63×63 symmetric connectivity matrix comprising the PLI values for each pair of EEG channels.

The whole-scalp PLI was computed as the average of all pairs of channels. To explore the spatial distribution of resting-state functional connectivity, we additionally computed region-specific PLI values by first clustering all channels into four regions of interest (ROIs), which were frontal, central, temporal, and parietooccipital scalp areas (see Fig. S1), and then averaging the PLI values of all pairs of channels within each ROI (e.g., for frontal: F1-F3, F2-F4, Fz-F1, etc.) and between each pair of ROIs (e.g., for fronto-central: F1-C1, F1-C3, Fz-Cz, etc.). This resulted in 10 groups of region-specific PLI values: fronto-frontal, fronto-central, fronto-temporal, fronto-parietooccipital, centro-central, centro-temporal, centro-parietooccipital, temporo-temporal, temporo-parietooccipital and parietooccipital-parietooccipital.

Resting-state functional connectivity was visualized with the BrainNet Viewer toolbox (Xia, Wang, & He, 2013). Values of the 63×63 matrix below 0.085 were set to zero. Next, the PLI values, indicating the strength of connectivity between channels, were plotted as line thickness (Fig. 3).

2.6 Statistical analyses

To test whether beta and gamma whole-scalp PLI significantly changed from pretest to posttest within each treatment group, the two-sided Wilcoxon signed-rank test was used; this non-parametric test was used because the PLI data were not normally distributed according to Shapiro-Wilk tests. The same test was also used to test for a gamma-PLI change from pretest to posttest within each treatment group for each of the 10 region-specific electrode groups. To test whether the whole-scalp gamma-PLI changes in the gamma stimulation group were significantly different from the gamma-PLI changes in the beta and sham stimulation groups, the two-sided Wilcoxon rank-sum test was used for between-subject comparisons. Rank correlations between functional connectivity change and the previously reported changes in CRS-R and gamma and beta ASSR and their significance were assessed using Spearman's correlation coefficient ρ . A significance criterion $\alpha = 0.05$ was used. Type-I error probabilities inflated by multiple comparisons were corrected by controlling the false-discovery rate (FDR) (Benjamini & Hochberg, 1995). Effect sizes for non-parametric statistical tests were quantified using the formula: $r = \frac{Z}{\sqrt{N}}$, where Z represents the test statistic and N is the sample size (Rosenthal, 1991).

3 Results

3.1 Positive aftereffect of gamma acoustic-electric stimulation on resting-state functional connectivity

To identify whether gamma stimulation modulates resting-state functional connectivity, we compared the DOC patients' whole-scalp PLI before vs. after gamma stimulation (pretest vs. posttest). We tested this for resting-state activity in the gamma frequency band and a control frequency in the beta band. We found a significant increase of whole-scalp PLI in gamma band ($Z = 3.011$, $p = 0.003$, $r = 0.444$, Fig. 2A, left), but no significant change in beta band ($Z = 1.947$, $p = 0.052$, $r = 0.287$, Fig. 2B, left).

Figure 3 visualizes the scalp topography of patients' resting-state functional connectivity in the gamma band. It can be seen that the strengthening of brain connectivity from pretest (Fig. 3A) to posttest (Fig. 3B) involves widespread regions over the scalp. To specify the particular brain regions in which the aftereffect on gamma PLI occurred, we segmented the scalp into four ROIs (Fig. S1) and tested the effect of gamma stimulation (pretest vs. posttest) on gamma PLI within each ROI and between each pair of ROIs (see more details in Methods). As shown by Figure 4, we found a significant increase of PLI both within the majority of ROIs and between all pairs of ROIs, with the largest changes in the frontotemporal region (frontal, central, temporal, parietooccipital ROIs and their pairings: $Z > 2$, $p < 0.05$, $r > 0.30$; except within frontal ROI: $Z = 1.916$, $p = 0.055$, $r = 0.282$, FDR corrected). Overall, these results suggest that acoustic-electric gamma TN stimulation strengthens gamma-band connectivity within and between widespread cortical regions.

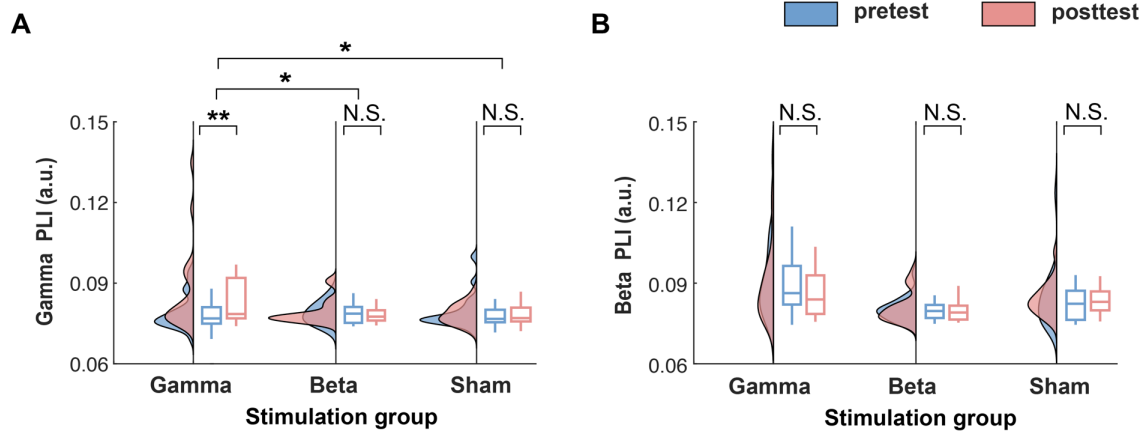


Figure 2. DOC patients' resting-state functional connectivity in the gamma and beta band before and after rhythmic acoustic-electric or sham TN stimulation.

(A). Whole-scalp gamma PLI in DOC patients in pretest (blue) and posttest (red). The leftmost pair of plots represents the group of patients who underwent gamma stimulation (gamma-stimulation group). The middle and rightmost pairs represent the patient group receiving beta stimulation (beta-stimulation group) and sham stimulation (sham-stimulation group), respectively. Only gamma stimulation was found to have a strengthening effect on patients' brain connectivity in the gamma band, which was significantly larger than the non-significant change in the beta and in the sham stimulation groups. (B). Same as (A), but for brain connectivity in the beta band. Neither acoustic-electric nor sham stimulation had an effect on patients' brain connectivity in the beta band. The raincloud plots visualize the data distribution, the horizontal line within each boxplot indicates the median value across participants; 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, * $p < 0.05$, ** $p < 0.01$.

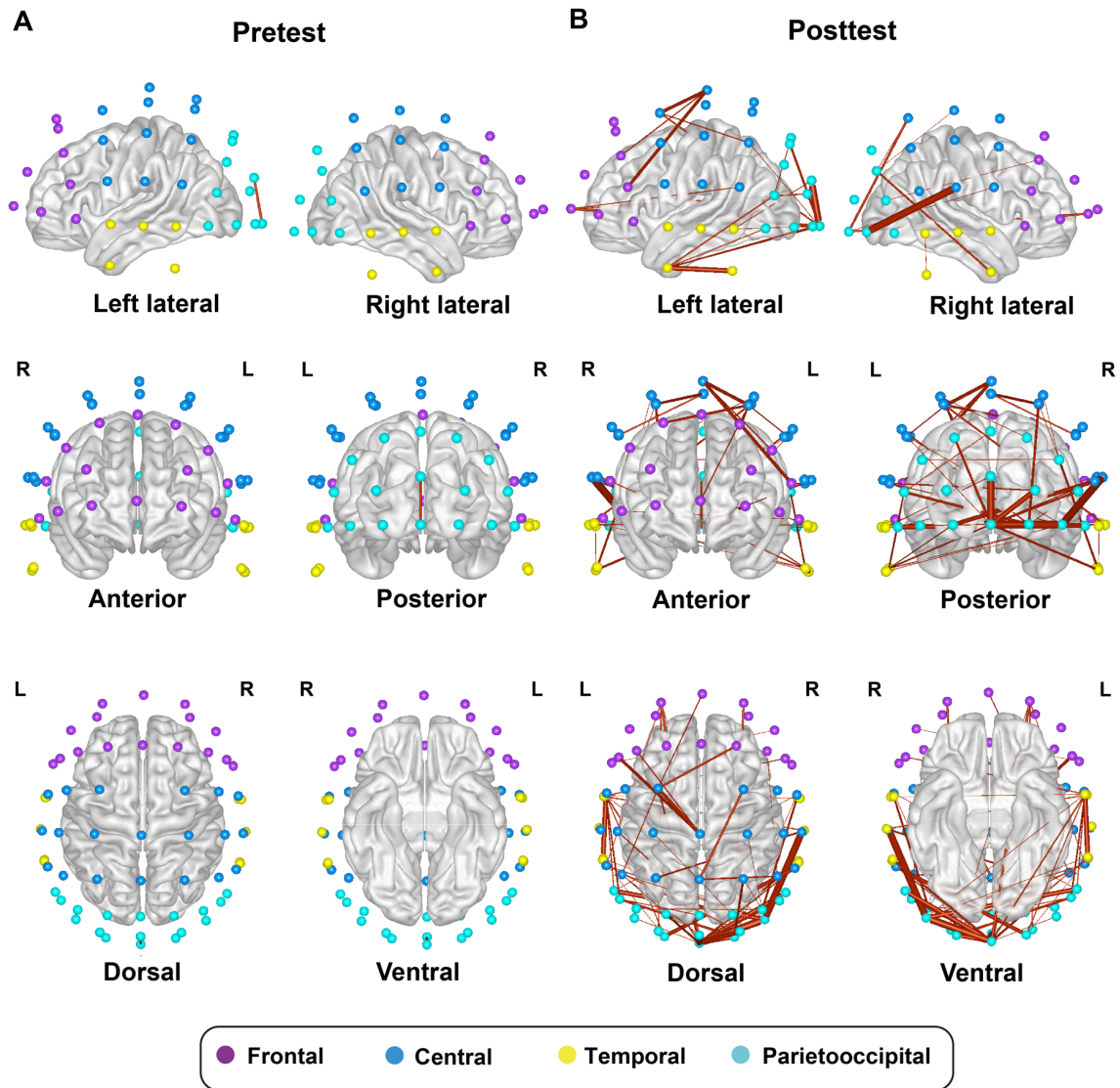


Figure 3. Scalp topography of DOC patients' gamma-band connectivity before and after gamma acoustic-electric TN stimulation. The spatial distribution of gamma-band connectivity is depicted in lateral (top row), anterior and posterior (middle row), and dorsal and ventral (bottom row) views of the cerebral cortex. The colored nodes represent the locations of the 63 EEG electrodes on the scalp. Purple, blue, yellow and cyan nodes respectively represent ROIs over the frontal, central, temporal and parietooccipital cortex. The red lines represent gamma-band connections between pairs of nodes. The line thickness represents the strength of the connection (with thicker lines representing higher PLI values, i.e., stronger connection). A threshold PLI value of 0.085 was applied for clear visualization. (A) Before gamma stimulation, DOC patients were observed to show almost no suprathreshold gamma-band connectivity within or between any ROIs. (B) After receiving the gamma stimulation, patients showed enhanced gamma-band connectivity within and between widespread ROIs.

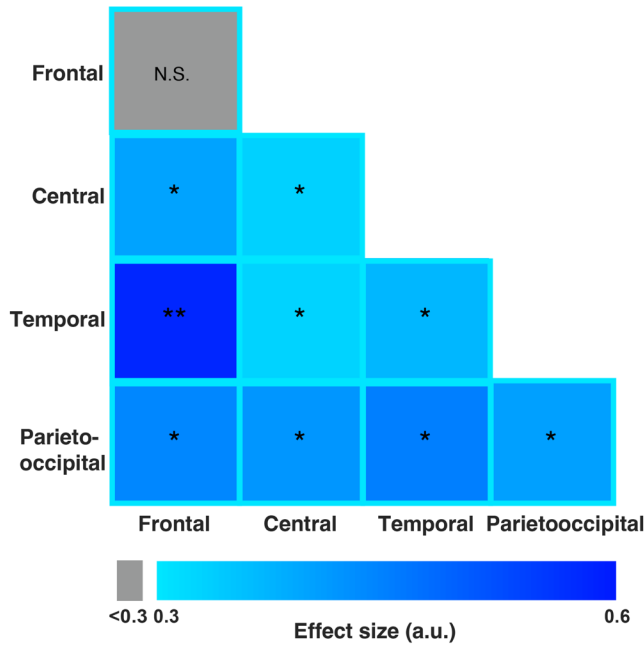


Figure 4. Effect of gamma acoustic-electric TN stimulation on DOC patients' gamma-band connectivity within and between regions of interest. Each cell represents the change of gamma-band connectivity within a given ROI (cells on the diagonal) or between a given pair of ROIs (cells off the diagonal) after gamma stimulation (pretest vs. posttest). The color of the cell represents the effect size of the change, with darker colors representing stronger PLI increases. Asterisks represent the statistical significance of the change (N.S. non-significant, $*p < 0.05$, $**p < 0.01$, FDR corrected). Gamma stimulation was found to significantly strengthen gamma-band connectivity both within ROIs and between each pair of ROIs.

3.2 No aftereffect of beta acoustic-electric stimulation on resting-state functional connectivity

To evaluate whether the positive aftereffect of rhythmic acoustic-electric stimulation on gamma-band connectivity generalizes from gamma stimulation to other (non-gamma) stimulation frequencies, we estimated PLI in another patient group receiving the rhythmic acoustic-electric stimulation at a control frequency in the beta band (i.e., 28 Hz). In contrast to the gamma-stimulation group, we found this beta-stimulation group to show no significant change of whole-scalp PLI in the gamma band (pretest vs. posttest: $Z = 0.075$, $p = 0.940$, $r = 0.012$, Fig. 2A, center). Moreover, this observed non-significant change was significantly smaller than the effect observed in the gamma-stimulation group (interaction stimulation frequency \times time, $Z = 2.240$, $p = 0.0248$, $r = 0.330$, Fig. 2A). Similar to the gamma-stimulation group, the beta-stimulation group showed no effect on resting-state functional connectivity in the control frequency band (beta PLI, $Z = 1.344$, $p = 0.179$, $r = 0.213$, Fig. 2B, center). The results from the gamma- and beta-stimulation groups together indicate that the observed strengthening of gamma-band connectivity was induced by the stimulation oscillating at gamma frequency, rather than the stimulation *per se*.

3.3 No aftereffect of sham acoustic-electric TN stimulation on resting-state functional connectivity

To further rule out that the strengthening aftereffect of gamma stimulation on gamma band connectivity was caused by potential spontaneous recovery and/or a placebo effect, we conducted

the experiment in another control group receiving sham stimulation (see Methods). Similar to the results obtained for the control group (see above), we found this sham-stimulation group to show no significant change of whole-scalp PLI from pretest to posttest (gamma PLI: $Z = 0.261$, $p = 0.794$, $r = 0.041$; beta PLI: $Z = 1.456$, $p = 0.145$, $r = 0.230$; Fig. 2, right) and the non-significant gamma PLI change was significantly smaller than the effect observed in the gamma-stimulation group (interaction stimulation type \times time: $Z = 2.118$, $P = 0.034$, $r = 0.312$; Fig. 2A). Thus, these results support the idea that the observed strengthening of gamma-band connectivity was induced by the gamma stimulation, rather than spontaneous recovery and/or a placebo effect.

3.4 Changes in gamma-band resting-state functional connectivity correlate with changes in auditory ability

To assess whether the observed changes in brain connectivity were potentially relevant for the patients' improvements in consciousness level as reported in (Wu et al., 2022), we next explored the correlation between patients' changes in whole-scalp gamma-band connectivity and their changes in CRS-R scores (posttest minus pretest). We found no significant correlation between gamma-band PLI changes and CRS-R total score changes ($p = 0.090$, $p = 0.241$, Fig. 5A). Further exploration of the six CRS-R sub-scales (auditory function, visual function, motor function, oromotor function, communication ability, and arousal) revealed a significant positive correlation of moderate strength between gamma-band PLI changes and changes of CRS-R score at the auditory sub-scale ($p = 0.224$, $p = 0.039$ [uncorrected], Fig. 5B). This observation of a putative link between gamma-band connectivity and auditory ability was further supported by a significant positive correlation between gamma-band PLI changes and ASSR changes at gamma and beta frequency (i.e., 28 Hz and 40 Hz, $p = 0.227$, $p = 0.041$, Fig. 5C). Moreover, the changes of gamma-band connectivity in the frontotemporal region that showed the largest change were also correlated to the changes of CRS-R auditory subscale and ASSR, but not the changes of CRS-R total score (CRS-R total score: $p = 0.164$, $p = 0.099$; auditory: $p = 0.209$, $p = 0.050$; ASSR: $p = 0.405$, $p = 0.001$, Fig. 5D-F). Thus, these neural-behavioral observations suggest that strengthening of gamma-band connectivity through gamma stimulation can potentially improve auditory processing abilities.

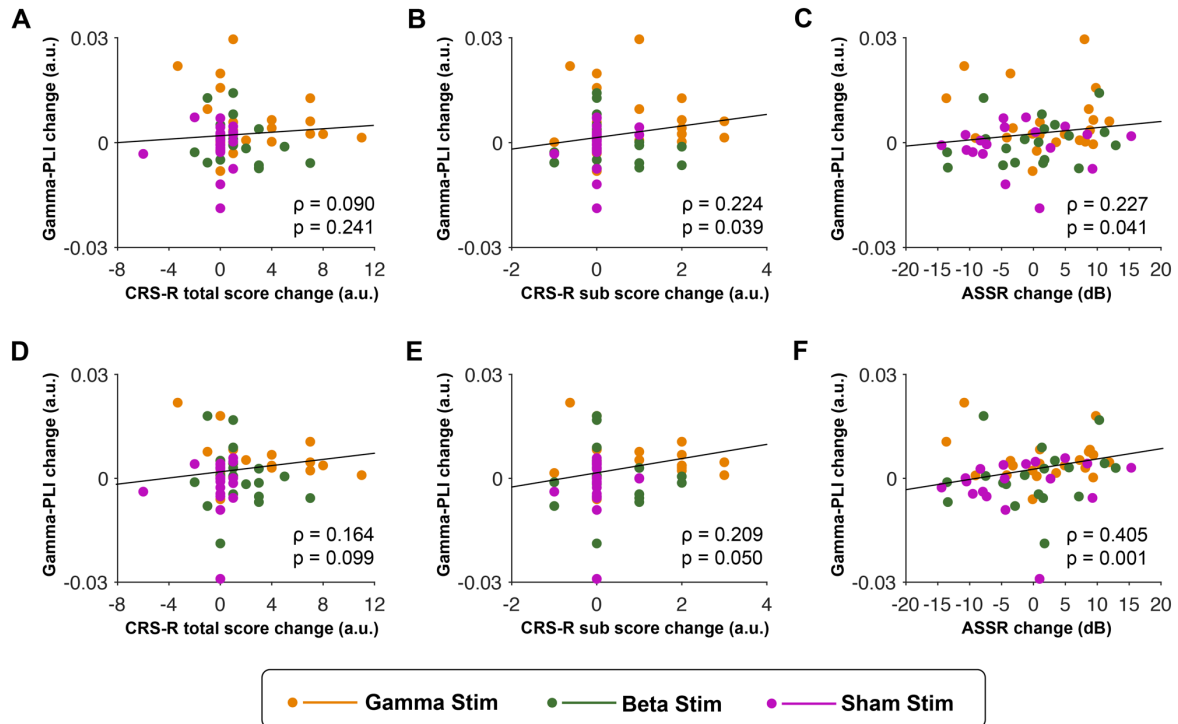


Figure 5. Correlation between changes in gamma-band resting-state functional connectivity and changes in the level of consciousness and auditory processing

(A). The scatterplot shows results from a correlation analysis testing for a functional coupling between changes in whole-scalp gamma-band connectivity and changes in CRS-R total score. (B). Same as (A), but for changes in CRS-R auditory sub-score (instead of changes in CRS-R total score). (C). Same as (A), but for changes in gamma and beta ASSR (instead of changes in CRS-R total score). (D-F). Same results as in A-C, but after limiting the analysis to the frontotemporal area that showed the largest gamma connectivity change. Correlation coefficient ρ and p -value describe, respectively, the strength and statistical significance of the coupling across all patients. Orange, green, and magenta dots respectively represent data points of individual patients in the gamma-, beta-, and sham-stimulation group.

4 Discussion

In this study, we found that gamma acoustic-electric TN stimulation significantly increased gamma-band resting-state functional connectivity in widespread areas across the cortex, while it did not affect beta-band connectivity. We found no such increase of gamma-band connectivity after beta stimulation or sham stimulation. Additionally, we observed that the gamma-band PLI changes correlated positively with changes of the ASSR and changes of the CRS-R auditory sub-scale, but not to changes of the CRS-R total score. Overall, these results show that gamma acoustic-electric TN stimulation can improve gamma-band resting-state functional connectivity, beyond potential effects of spontaneous recovery, placebo, or the mere presentation of sensory stimulation *per se*. They further suggest that the improvement of gamma-band resting-state functional connectivity could support auditory abilities in DOC patients.

4.1 *Rhythmic acoustic-electric TN stimulation increased resting-state functional connectivity in DOC patients*

TN stimulation has been an effective neuromodulation treatment for attention-deficit/hyperactivity disorder, migraine, and some other neurological disorders (DeGiorgio et al., 2013; McGough et al., 2019; Stanak, Wolf, Jagoš, & Zebeholzer, 2020). However, its application to DOC patients has remained limited. Fan et al. reported a significant change in degree centrality (a measure of global brain connectivity (Zuo et al., 2012)) in several brain regions (e.g., cerebellum and inferior temporal gyrus) after TN stimulation treatment in a single patient (Fan et al., 2019), thereby providing first fMRI evidence for the effect of TN stimulation on brain connectivity of a DOC patient. In our previous study (Wu et al., 2022), we found increased ASSR of DOC patients after acoustic-electric TN stimulation. Our current findings add to these previous findings, which together provide neurophysiological evidence that gamma acoustic-electric TN stimulation can induce effects on various aspects of cortical activity in DOC patients.

Despite these observations, how exactly gamma acoustic-electric TN stimulation, as a novel intervention approach for DOC, affects functional brain connectivity remains unclear. A candidate mechanism underlying the effects of acoustic-electric TN stimulation on resting-state functional connectivity may relate to the activation of the thalamus and the thalamocortical pathway. A recent study in rats in an unconscious state induced by traumatic brain injury has illustrated that gamma TN stimulation activates the lateral hypothalamus and upregulates neuropeptide hypocretin, which are beneficial for promoting consciousness recovery (Zheng et al., 2021). Moreover, direct electrical stimulation of the thalamus has been shown to improve consciousness and functional connectivity between the thalamus and the cortex (Redinbaugh et al., 2020; Schiff, 2009). Similarly, our rhythmic acoustic-electric TN stimulation was designed to induce strong synchronous activity in multisensory thalamic nuclei and thus restore connectivity between the thalamus and hierarchically higher structures in the cortex. However, this interpretation in terms of neural synchrony in multiple sensory thalamocortical pathways requires further verification in future research using methods that can spatially resolve thalamic and cortical activity, such as fMRI.

4.2 *Gamma acoustic-electric TN stimulation increased widespread connectivity*

We found that the increase of gamma-band connectivity after gamma acoustic-electric TN stimulation is not restricted to a specific region but involves the whole brain. This widespread nature of the effect suggests that gamma stimulation facilitates communication and integration of information across the entire cortex. This notion is supported by a recent study reporting that TNS activates widespread brain areas in mouse models with traumatic brain injury (Yang et al., 2022). It should be kept in mind that we estimated connectivity here at the level of scalp EEG electrodes. Thus, although the PLI may be less susceptible to the influence of volume conduction than other connectivity measures, the signal recorded by each sensor is still a mixture of the activity of different brain sources, which limits the conclusions that can be drawn about the spatial origin of the effects.

4.3 *The effect of acoustic-electric TN stimulation on brain connectivity is frequency-specific*

Interestingly, we observed that gamma, but not beta, acoustic-electric TN stimulation significantly increased brain connectivity, and the effects of gamma stimulation on connectivity were specific to the gamma band and did not generalize to the beta band. These findings highlight that the effects of rhythmic stimulation are frequency-specific, with a special susceptibility of gamma oscillations for gamma acoustic-electric stimulation.

External rhythmic stimulation at a given frequency may have the most substantial effect on brain circuits that have a matching resonance frequency. The thalamus has been postulated to

regulate arousal via thalamocortical synchronization at 30-40 Hz (Steriade, 2006) and stimulating the central thalamus at 40 Hz has been shown to cause widespread brain activity and arousal (Liu et al., 2015; Redinbaugh et al., 2020). Thus, the frequency specificity of thalamocortical loops suggests that the most effective stimulation frequency is in the gamma band, particularly around 40Hz. The fact that our scalp EEG results show widespread connectivity changes and gamma-frequency specificity suggests that these effects involve thalamocortical loops

4.4 *Enhanced brain connectivity is related to improved auditory abilities*

In contrast with our original hypothesis, we did not find a significant correlation between the change in gamma band connectivity and the change in patients' global consciousness state, as measured by the CRS-R total scores, suggesting the observed global improvement in consciousness is not necessarily accompanied by a gamma-band connectivity change. Thus, it seems that the gamma-band connectivity increase is not the mechanism underlying the observed global improvement in consciousness. This interpretation conflicts a previous study by Naro and colleagues (Naro, Bramanti, et al., 2018), which demonstrated a significant correlation between CRS-R total score and connectivity at gamma frequency in DOC patients (Naro, Bramanti, et al., 2018). The difference to the previous results may reflect methodological differences: Naro et al. used a different estimator of functional connectivity (dwPLI, a debiased estimator of the squared weighted PLI), retained only the top 30% of dwPLI values, defined gamma band as 25-40 Hz, and correlated CRS-R score and dwPLI at a specific point in time, while we used PLI, analyzed all PLI values, defined the gamma band as the range from 30-45 Hz, and correlated *changes* between pretest and posttest.

However, we found significant correlations between the gamma-band connectivity change and changes in the CRS-R auditory subscore as well as changes in the ASSR. ASSRs – phase-locked responses to amplitude-modulated auditory stimuli – are commonly used as an objective measure of hearing sensitivity (Galambos, Makeig, & Talmachoff, 1981). These results suggest a link between gamma-band connectivity and auditory processing. Gamma-band synchronization has been considered as a mechanism for interregional communication that relates to sensory processing and a variety of cognitive processes (Basar, 2013; Misselhorn, Schwab, Schneider, & Engel, 2019). For instance, an increase of gamma-band functional connectivity between auditory cortices and the anterior cingulate cortex was observed during an auditory detection task (Leicht et al., 2021; Steinmann et al., 2014). In line with these studies, our results suggest that increased gamma connectivity is coupled with improvement of auditory abilities. Moreover, the largest gamma connectivity change, which was observed in frontotemporal regions, also correlated to the changes of CRS-R auditory subscale or ASSR. Thus, these results suggest that gamma-band connectivity, particularly in frontotemporal regions, might underlie the observed improvement in auditory processing and auditory abilities.

Although from the correlation results we cannot conclude that gamma connectivity is the mechanism underlying the consciousness improvement, the observed coupling between the changes of gamma connectivity and the changes of CRS-R auditory subscale or ASSR suggests that facilitation of gamma connectivity might underlie the improvement in a specific aspect of consciousness, that is the auditory abilities. Note that the correlation results were not corrected for multiple comparisons; therefore, our interpretation of these results remains tentative and requires further confirmation.

4.5 *Conclusions*

In sum, this study illustrates that gamma oscillatory acoustic-electric TN stimulation can support functional brain connectivity across the cortex and that the observed change in gamma connectivity may play an important role in auditory processing. These findings provide critical insight into the

neural effects of the gamma acoustic-electric TN stimulation approach, which is essential for its clinical translation into a potential novel treatment approach.

Abbreviations

AM = Amplitude modulation; ASSR = auditory steady-state response; CRS-R = Coma Recovery Scale-Revised; DOC = disorders of consciousness; FDR = false-discovery rate; fMRI = functional magnetic resonance imaging; IIR = Infinite Impulse Response; MCS = minimally conscious state; PLI = phase-lag index; ROIs = regions of interest; TN = trigeminal nerve; UWS = unresponsive wakefulness syndrome

Data availability statement

Data will be made available on request.

Ethical approval and consent to participate

The study was approved by the ethics committee of Hangzhou Mingzhou Brain Rehabilitation Hospital (2020003) and was carried out in accordance with the Declaration of Helsinki. Informed consent of all enrolled patients was obtained from the patients' legal surrogates prior to the study.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (81870817) to B.L. and China Scholarship Council to M.W. (CSC 201906320078).

Conflict of Interest

All authors declare no competing interests.

Author Contributions

Min Wu: Conceptualization, Methodology, Data curation, Formal analysis, Writing – original draft, Writing – review & editing; **Marta Concolato:** Methodology, Writing – original draft, Formal analysis, Writing – review & editing; **Bettina Sorger:** Conceptualization, Supervision, Writing – review & editing; **Yanmei Yu:** Data collection, Writing – review & editing; **Xiaxia Li:** Data collection, Writing – review & editing; **Benyan Luo:** Conceptualization, Funding acquisition, Data curation, Writing- review & editing; **Lars Riecke:** Conceptualization, Methodology, Supervision, Writing – original draft, Writing – review & editing.

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5 Supplementary materials

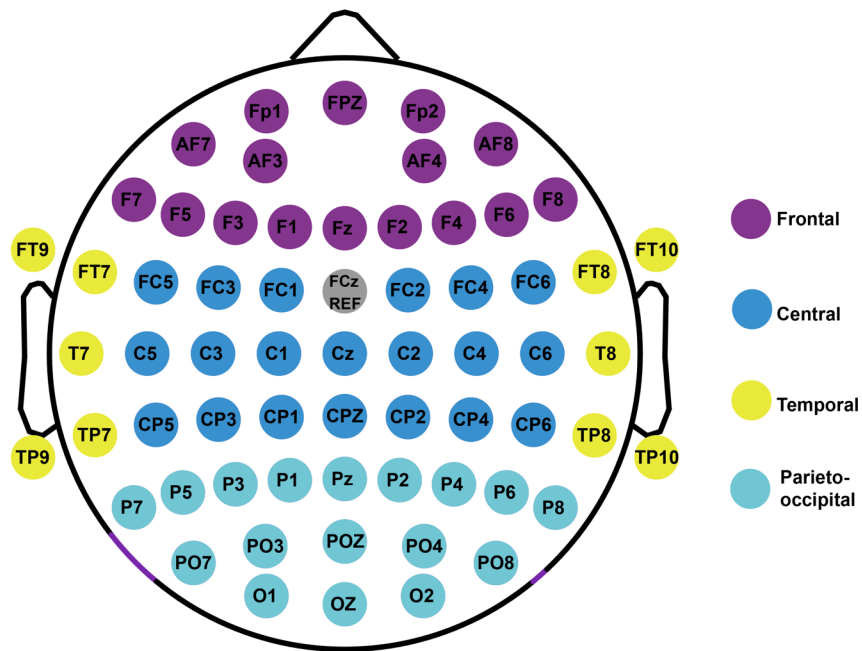


Figure S1. Segmentation of the scalp into four regions.

Scalp locations were clustered into four regions of interest: frontal, central, temporal, and parietooccipital regions (represented by the different hues in the figure). Functional connectivity was assessed within each ROI and between each pair of ROIs.

Chapter 6

General discussion

In this dissertation, we developed a novel rhythmic acoustic-electric stimulation approach and systematically evaluated its effectiveness in modulating consciousness. In Chapter 2, we modified a consciousness assessment paradigm by incorporating amplitude modulation with a speech paradigm. The neural responses to amplitude-modulated speech waveforms and hierarchical temporal structures of speech enabled the assessment of oscillatory brain activity and speech-structure tracking. In Chapter 3, we developed an innovative rhythmic acoustic-electric stimulation approach and investigated its effectiveness in modulating conscious perception in healthy participants. This novel stimulation approach was further evaluated in a patient population with disorders of consciousness (DOC), as described in Chapter 4. In Chapter 5, we explored the modulatory effects of the stimulation approach on resting-state functional brain connectivity, thereby providing insight into the underlying neurophysiological mechanisms.

1 Summary

In Chapter 2, our objective was to estimate multiple neurophysiological responses and explore word-processing dynamics during a speech paradigm. As introduced in Chapter 1, semantic processing, as measured by the higher-level speech-structure tracking, can serve as indicators for consciousness. We presented healthy human participants with auditory stimuli consisting of isochronous word sequences arranged into either predictable (coherent sentences) or less predictable (unstructured, random word sequences) 42-Hz amplitude-modulated speech, accompanied by a continuous 25-Hz amplitude-modulated distractor tone. We recorded neural responses to hierarchical linguistic structures, and reaction times and frequency-tagged neuroelectric responses (auditory steady-state responses, ASSRs) to individual words at various temporal positions within the sentences. We observed spectral peaks at the sentence rate, its harmonics, and the word rate in the structured speech condition, indicating successful tracking of sentence and word structures. Moreover, we observed prominent ASSRs at tagging frequencies (25 Hz and 42 Hz), reflecting phase-locked cortical responses to the amplitude-modulated speech and tone. By quantifying responses at each position while controlling for individual word characteristics (phonetics, frequency, and familiarity), we observed that sentential context increasingly facilitates auditory word processing. These word-processing dynamics occurred only when listeners focused their attention on the speech and did not transfer to the auditory processing of the concurrent distractor tone. These findings suggest that auditory word-processing dynamics during sentence tracking can originate from sentential predictions, which rely on the listener's attention to the speech and only influence the processing of parsed speech, not concurrently presented auditory streams. Our findings suggest that this speech paradigm can be used to track multiple levels of linguistic processing, oscillatory brain activity, and word-processing dynamics. We used a simplified version of this speech paradigm for assessing consciousness in patients with DOC in Chapter 4.

In Chapter 3, we investigated the effectiveness of gamma-band multimodal stimulation that combines musical stimulation and electrical trigeminal nerve stimulation (TNS), in enhancing conscious perception. We evaluated healthy human participants' conscious perception before and after stimulation using behavioral and neural measures in tactile and auditory target-detection tasks. We observed that the acoustic-electric stimulation improved conscious tactile perception without affecting auditory perception. This improvement was attributed to the combination of acoustic and electric stimulation, instead of either unimodal stimulation alone. To investigate the underlying mechanisms of acoustic-electric stimulation, we further fitted a biologically plausible neural network model to the neural data using dynamic causal modeling (DCM). Our modelling findings revealed that acoustic-electric stimulation may have exerted a modulatory effect on a somatosensory recurrent neural processing network. Alterations in inter-regional connections within the network were found to correlate with the changes in conscious perception after stimulation. These findings support the

use of acoustic-electric stimulation to promote conscious perception and confirm the value of the stimulation. The beneficial effects of acoustic-electric stimulation on consciousness may be due to the modulations in inter-regional cortical connections.

In Chapter 4, we further investigated the clinical translatability of the novel acoustic-electric stimulation approach in patients with DOC. We applied the stimulation in a large sample of DOC patients for 40 min on five consecutive days. We measured patients' consciousness before and after the stimulation using behavioral scales and neural responses in an adapted version of the auditory speech paradigm developed in Chapter 2. We found that acoustic-electric stimulation in gamma and beta frequencies reliably led 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. Patients with stronger behavioral benefits showed stronger improvements in oscillatory brain activity. Moreover, 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 (assessed with the communication subscale) and their semantic violation-detection ability (assessed with event-related potentials to semantically incongruent words in the speech paradigm), but no systematic effect on speech tracking (assessed with spectral peaks, introduced in Chapter 2). These findings suggest that acoustic-electrical stimulation may represent a promising approach to improve certain aspects of consciousness in patients with DOC and that oscillatory brain activity may contribute to consciousness.

In Chapter 5, we analyzed neural and behavioral data from patients with DOC who underwent five days of gamma, beta, or sham acoustic-electric stimulation. Resting-state electroencephalography (EEG) was measured before and after the stimulation, and resting-state functional connectivity was evaluated using the phase-lag index (PLI). We found that gamma stimulation induces an increase of PLI in the gamma band. Further characterization revealed that the enhancing effect is specific to the gamma band, as we observed no comparable change in beta-band PLI and no effect of beta-band acoustic-electric stimulation or sham stimulation. The PLI changes in the gamma band were widely spread across the cortex, with the largest alterations in the frontotemporal region, and were accompanied by improvements in patients' auditory abilities. These findings thus show that gamma-band acoustic-electric stimulation can improve resting-state functional connectivity in the gamma band, which in turn may be linked to auditory abilities and/or consciousness restoration in DOC patients.

2 Speech paradigm and its implications

2.1 *Sentential contextual facilitation on auditory word processing*

We observed that sentential context can progressively facilitate word processing as the sentence unfolds, reflected as a gradual decrease in reaction times and an increase in auditory steady-state responses to words towards the end of sentences. The sentential contextual facilitation in reaction times can be explained as follows: as a sentence unfolds, the semantic context incrementally builds up, which increases the predictability of later words in the sentence (Lam, Schoffelen, Udden, Hulten, & Hagoort, 2016; Marslen-Wilson & Tyler, 1975; Van Petten & Kutas, 1990, 1991). Consequently, listeners may have recognized later words more quickly than earlier words because their meanings were more predictable. Regarding the neural measures, our measure of auditory steady-state responses reflects phase-locked responses to artificially-induced rapid modulations of the word waveform (amplitude modulation) (Kuwada, Batra, & Maher, 1986; Picton, John, Purcell, & Plourde, 2003; Rodriguez, Picton, Linden, Hamel, & Laframboise, 1986). Therefore, the neural results may indicate a primary effect of sentential predictability on cortical processing of lower-level acoustic

speech-signal features. Overall, they strongly suggest that the sentential context affects both lower-level acoustic and higher-level speech processing.

We also observed that sentential contextual facilitation in auditory word processing occurs only when listeners pay selective attention to speech. Previous findings have shown that attention is necessary for listeners to group syllables into words (Ding et al., 2018; Makov et al., 2017). Our results extend these findings by demonstrating that listeners can group words into sentences and extract contextual information only when they pay attention to speech, which consequentially contributes to the word-processing dynamics. Furthermore, we found that sentential contextual facilitation does not always transfer to the processing of concurrent sound streams. Previous research has shown that perceptual segregation may occur as early as the cochlear nucleus (Pressnitzer, Sayles, Micheyl, & Winter, 2008). Therefore, the perceptual segregation of concurrent acoustic streams from speech likely precedes the sentential contextual facilitation of acoustic word processing (Alain, Arsenault, Garami, Bidelman, & Snyder, 2017; Bidelman & Alain, 2015; Bidelman & Yellamsetty, 2017), resulting in no facilitation observed in concurrent tone streams.

As word-processing dynamics rely on semantic processing and attentional abilities while do not require overt behavioral responses, we hypothesize that these dynamics may serve as a signature for consciousness. Specifically, this approach could be used to identify individuals with cognitive motor dissociation, who show neuroimaging evidence of consciousness but lack detectable command-following behaviors (Schiff, 2015). Although further exploration and validation are required for its scientific and clinical applications, this method holds promise for uncovering covert consciousness.

2.2 Speech tracking, semantic deviation detection

Compared to findings in healthy participants that showed significant sentence, phrase and word tracking (Chapter 2), we observed word tracking in all DOC patients, and sentence and phrase tracking only in a subgroup of patients (Chapter 4). These results are consistent with those of previous research (Gui et al., 2020; Sokoliuk et al., 2021). Despite the fact that we simplified the speech stimuli by using repetitive word quartets rather than various sentences, DOC patients failed to reliably track sentence and phrase structures, showing their limited level of consciousness.

We further estimated the N400 component evoked by semantically congruent words vs. semantically incongruent words to assess neural detection of semantic violations. However, we did not observe N400 reliably across patients or sessions. Together with the speech tracking results, these findings indicate that DOC patients do not reliably preserve speech processing ability, which includes both sentence tracking and semantic violation detection.

Furthermore, we assessed the effects of acoustic-electric stimulation on speech tracking and semantic violation detection. We found that the stimulation had no systematic effect on the neural tracking of words or sentences. However, we found patients could detect semantic violations after gamma-band acoustic-electric stimulation, as reflected by a visible negative N400 response. 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. As introduced in Chapter 1, detection of semantic violations (i.e., N400) has been observed during sleep, although with lower strength than during wakefulness (Ibanez, Lopez, & Cornejo, 2006). In contrast, neural tracking of sentential structure is not observable during sleep and in UWS patients (Makov et al., 2017). This implies that detection of semantic deviations reflects a lower-level automatic process (Ibanez et al., 2006; Kiefer, 2002), whereas tracking sentential structure relies on continuous comprehension using top-down lexical knowledge (Ding et al., 2018). Therefore, it is possible that our gamma stimulation affects only lower-level speech processing, while leaving higher-order speech comprehension unaffected.

In conclusion, sentence tracking is thought to represent a high-level cognitive ability, potentially exhibiting high specificity but low sensitivity for consciousness detection and assessments of treatment efficiency. Comparatively, N400 may represent an automatic process, which is speculated to have higher sensitivity than sentence tracking. Additionally, as speech processing represents only one aspect of consciousness (Bayne, Hohwy, & Owen, 2016), assessing other aspects and using a combination of multiple neurophysiological indicators are recommended to ensure an accurate and comprehensive evaluation of consciousness and treatment effects in DOC patients.

3 Rhythmic acoustic-electric stimulation

3.1 Trigeminal nerve stimulation and consciousness

In Chapters 3 and 4, we observed that multimodal acoustic-electric stimulation, which combines musical stimulation and electrical trigeminal nerve stimulation, can enhance tactile perception in healthy participants and improve the level of consciousness in patients with DOC. However, given that this stimulation approach is still relatively novel, the mechanism of its action on consciousness has been unclear. Here, we suggest a number of potential candidate mechanisms based on existing evidence.

The “mesocircuit hypothesis” model provides a neuronal framework for understanding the mechanisms underlying consciousness recovery (Schiff, 2010, 2022). This model highlights the central role of the thalamus in consciousness, along with frontal cortical regions and the striatum, which together form the frontocortico-striatopallidal-thalamocortical loop system. Severe brain injury can lead to the loss of excitatory thalamocortical and thalamostriatal projections, subsequently resulting in the loss of consciousness. Moreover, the accompanying loss of projection from cortical and thalamus to the neurons in striatum leads to the release of tonic firing of pallidal neurons within the globus pallidus interna. This, in turn, leads to the active inhibition of central thalamus (Schiff, 2010, 2022). As discussed in Chapter 1, the anatomy of the trigeminal nerve enables TNS to stimulate the thalamus and activate its projections to the cortex. According to the mesocircuit model, this stimulation could facilitate the restoration of thalamocortical and thalamostriatal connections, thereby promoting the recovery of consciousness.

Furthermore, a “vagal cortical pathway model” has been proposed to explain the action of vagal nerve stimulation, which consists of four consecutive pathways (Briand, Gosseries, Staumont, Laureys, & Thibaut, 2020). Firstly, this model involves the activation of the trigeminal nucleus and the tractus of the solitarius nucleus located in the lower brainstem (Kerr, 1961). Next, this activation leads to the activation of the locus coeruleus and the raphe nuclei localized in the upper brainstem (Grzanna & Fritschy, 1991; Jean, 1991). Then, the locus coeruleus produces norepinephrine and modulates global activity in many brain areas, such as thalamus and frontal cortex (Cao, Lu, Powley, & Liu, 2017). Finally, the raphe nuclei produce serotonin and modulate some structures of the limbic system and the frontal cortex (Giorgi et al., 2017). Inspired by this model, we propose the analogous “trigeminal cortical pathway model”. In this model, the trigeminal nerve directly projects to the trigeminal nucleus, with reciprocal projection to the locus coeruleus and raphe nuclei (Capra & Dessem, 1992), forming a pathway similar to that of VNS. Therefore, akin to VNS, TNS can lead to the activation of both the locus coeruleus and raphe nuclei, enhancing the release of norepinephrine and serotonin, and thereby regulating arousal (Mokler et al., 1998; Samuels & Szabadi, 2008).

Moreover, TNS has been shown to influence the autonomic nerve system. In the case of hemorrhagic shock, research shows that TNS induces strong synergistic coactivation of the sympathetic and parasympathetic nervous systems as indicated by heart rate variability. As a result, TNS has been proposed as a novel resuscitation strategy (Li et al., 2019). Additionally, this autonomic nerve regulation has been observed in cerebral blood flow (White et al., 2021). Although some animal

studies showed TNS can modulate the blood-brain-barrier, it remains unclear whether such findings can be translated from animal models to human patients, given the significant differences in brain structure and function. Therefore, more research is still needed to explore the mechanisms of TNS on consciousness improvements in humans.

3.2 Multimodal stimulation and consciousness

In Chapter 3, we observed that the effects of acoustic-electric stimulation on conscious tactile perception result from the interplay of the acoustic and electric stimulation rather than any of the unimodal stimulation alone. The effects of multimodal stimulation were further validated in DOC patients, as shown in Chapter 4.

One potential explanation for the unique impact of multimodal stimulation could be simple additive effects of unimodal acoustic and electric stimulation. However, this interpretation is difficult to reconcile with our observation of non-significant effects of either unimodal acoustic stimulation or unimodal electric stimulation (Chapter 3), making this alternative less plausible. It is important to note that the effects of acoustic-electric stimulation and electric-only stimulation were tested in separate groups, which may result in differences in sensitivity between participant groups. This could be a contributing factor to the observed null effects in the electric-only stimulation group. Nonetheless, comparing these two stimulation modalities within the same group is challenging, as muting the acoustic stimulation could unblind the participants, potentially introducing bias into the study.

Instead, a more intriguing explanation is that the consciousness benefits were driven by the integration of the acoustic and electric inputs. This notion has been supported by animal studies. For example, auditory-tactile integration has been found in the primary auditory cortex of monkeys (Lakatos, Chen, O'Connell, Mills, & Schroeder, 2007). Furthermore, animal studies in mice have shown that auditory stimulation and forepaw tactile stimulation can activate neurons in the somatosensory cortex (Godenzini et al., 2021). When the tactile stimulation was paired with the auditory stimulation, neuronal activation in somatosensory cortex and behavioral performance in a tactile-based task were significantly improved. These results indicate that the somatosensory cortex can encode multisensory information, and auditory input to it can enhance its sensory encoding, leading to changes in responses to tactile input.

Additionally, in vivo studies have provided further evidence of multimodal integration at the cellular level. For instance, auditory stimulation was shown to evoke postsynaptic spikes, and somatosensory stimulation was shown to elicit presynaptic activity in fusiform cells. The combination of the acoustic and electric stimulation leads to a significantly increased spike activity and produces long-term changes in fusiform cell firing rates (Marks et al., 2018; Wu, Stefanescu, Martel, & Shore, 2015). Similarly, when auditory stimulation was paired with a tactile stimulus, a significant increase in evoked firing rate occurred compared to the tactile stimulus alone in the dendrites of layer 2/3 pyramidal neurons in somatosensory cortex (Godenzini et al., 2021). These findings suggest that the interaction between multiple sensory modalities can facilitate neural firing and modulate spike-timing dependent plasticity (STDP), thereby contributing to the benefits of multimodal stimulation on behavioral performance.

Besides the STDP mechanism, neural entrainment, which synchronizes instinct neural oscillations with the externally applied stimulation, is another main mechanism underlying rhythmic stimulation effects (Lakatos, Gross, & Thut, 2019; Thut, Schyns, & Gross, 2011; Vogeti, Boetzel, & Herrmann, 2022). Although existing evidence shows that neural entrainment plays a major role in online effects, it remains possible that it partially contributes to the offline effects of rhythmic stimulation (Vogeti et al., 2022; Vossen, Gross, & Thut, 2015). Our findings in patients with DOC support this notion, as indicated by increased oscillatory brain activity after rhythmic stimulation.

However, the significance of synchronization between acoustic and electric stimulation for consciousness benefits remains unclear. Altering synchronization may reduce neural entrainment, but may not necessarily impact STDP (Vogeti et al., 2022). Nevertheless, addressing this question is challenging within the scope of this dissertation, as we have not manipulated the phases between acoustic and electric stimulation.

Given these findings, multimodal stimulation might be more effective than unimodal stimulation. However, we need to be cautious in concluding the ineffectiveness of unimodal electric stimulation, particularly in patients with DOC. In many transcranial direct current stimulation studies, unimodal electric stimulation has been shown to produce significant effects on consciousness in DOC patients (Angelakis et al., 2014; Thibaut, Bruno, Ledoux, Demertzi, & Laureys, 2014; Thibaut et al., 2017). It is possible that applying stimulation at higher intensities and specific frequencies, conducting multiple sessions, or combining different types of electric stimulation may elicit similar or enhanced effects on consciousness.

3.3 *Gamma frequency and consciousness*

We found that acoustic-electric stimulation at gamma frequency modulates consciousness in both healthy participants and patients with DOC. Moreover, our findings demonstrated a frequency-specific modulation of consciousness, with more pronounced effects on consciousness, oscillatory brain activity (Chapter 4) and brain connectivity (Chapter 5) observed at gamma frequency than at beta frequency. These findings suggest that acoustic-electric stimulation modulates consciousness in a frequency-specific manner, with larger effects at gamma frequency.

The improvements of consciousness after gamma stimulation might be attributed to the observed increase in gamma brain activity. This interpretation aligns with ideas from a previous non-invasive human brain-stimulation study showing a similar pattern of behavioral and neural changes. In that study, a positive frequency-specific effect of 25 Hz and 40 Hz transcranial alternating current stimulation on oscillatory brain activity and consciousness during dreaming was found and interpreted as a causal role of frequency-specific brain activity for consciousness (Voss et al., 2014). Previous studies showed that direct electrical stimulation of the thalamus may improve consciousness (Rezaei Haddad, Lythe, & Green, 2019; Schiff, 2008; Schiff et al., 2007). Based on this, 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 from patients' thalamus and/or its interaction with hierarchically higher structures in the cortex.

Interestingly, our results revealed a frequency-specific effect of acoustic-electric stimulation, with more pronounced improvements in consciousness observed after gamma stimulation compared to beta stimulation. The frequency-specificity may be attributed to the resonance properties of brain structures. More specifically, brain circuits tend to resonate at specific frequencies, and external rhythmic stimulation at a matching frequency can have the most substantial impact on these circuits (Lakatos et al., 2019; Vogeti et al., 2022). In the context of consciousness, the thalamus has been postulated to modulate and maintain conscious states via thalamocortical synchronization at 30-40 Hz (Steriade, 2006), and stimulating the central thalamus at 40 Hz has been shown to cause widespread brain activity and arousal (Liu et al., 2015; Redinbaugh et al., 2020). Furthermore, synchronization of 40 Hz oscillations between the prefrontal and parietal human cortex has been observed during conscious perception (Desmedt & Tomberg, 1994). This synchronization of neural activity represents a functional "binding" of relevant perceptual features, facilitating the integration of information and enabling conscious perception. The frequency specificity of thalamocortical loops and prefrontoparietal connectivity suggests that the most effective stimulation frequency lies within the gamma band, particularly around 40 Hz. By targeting the most effective frequency for specific brain circuits, we can potentially enhance the therapeutic effects of stimulation for patients with DOC. This finding highlights the importance of considering frequency specificity in the development and

application of acoustic-electric stimulation treatments, as it may be important in determining their effectiveness in modulating consciousness.

4 Brain connectivity

We observed that acoustic-electric stimulation in healthy participants can modulate connection strength within a recurrent neural processing network (Chapter 3). The enhanced conscious tactile perception was accompanied by connectivity changes within this network, i.e., increased bottom-up excitation from the contralateral primary somatosensory cortex to the contralateral secondary somatosensory cortex, and reduced top-down inhibition from the ipsilateral premotor cortex to secondary somatosensory cortex. These connectivity changes may serve to optimize the balance between excitation and inhibition, thereby enhancing sensory processing and perception.

In DOC patients, we observed that gamma acoustic-electric stimulation increased widespread resting-state functional connectivity (Chapter 5). A potential mechanism underlying these effects may involve the activation of the thalamus and thalamocortical pathway. A recent study in rats with unconsciousness induced by traumatic brain injury has illustrated that gamma TNS activates the lateral hypothalamus and upregulates neuropeptide hypocretin, which are beneficial for promoting consciousness recovery (Zheng et al., 2021). Similarly, our stimulation combining acoustic stimulation and electrical TNS was designed to induce strong synchronous activity in multisensory thalamic nuclei, restoring connectivity between the thalamus and higher cortical structures.

The distinct patterns of connectivity changes in healthy participants and patients with DOC were notable and several reasons could potentially account for these differences. Firstly, healthy individuals have intact neural networks, and the observed connectivity changes may primarily serve to optimize sensory processing and perception. In contrast, DOC is characterized by disrupted neural networks, and the connectivity changes in these patients may represent attempts to restore functional communication between brain regions and improve overall neural functioning (Bodien, Chatelle, & Edlow, 2017; Boly et al., 2011; Laureys et al., 2000; Laureys et al., 1999). Thus, differences in baseline neural functioning between the two participant groups may have contributed to the observed effects.

Additionally, methodological differences between the two studies could contribute to the observed differences. Firstly, performing tasks as opposed to being in a resting state engaged distinct brain networks (Cole, Bassett, Power, Braver, & Petersen, 2014; Fox et al., 2005; Smith et al., 2009). In healthy participants, connectivity was assessed during a tactile task, which involves networks required for the specific demands of the task, whereas connectivity in patients with DOC was measured during a resting state, involving resting-state connectivity representing intrinsic, default brain organization.

Secondly, the connectivity analyses employed different techniques: DCM for healthy participants and PLI for patients with DOC. DCM is a hypothesis-driven approach that infers effective connectivity between predefined brain regions based on observed neural activity (Stephan et al., 2010). In our healthy participants study, DCM allowed us to investigate the modulation of connectivity within the recurrent processing model, which included specific brain structures but not the entire brain. In contrast, PLI is a data-driven measure of synchronization between brain regions that quantifies the consistency of phase differences between pairs of neural signals without providing information about connection directionality (Stam, Nolte, & Daffertshofer, 2007). In our study with DOC patients, PLI enabled us to assess changes in functional connectivity across the whole brain without making assumptions about directionality or requiring predefined regions of interest.

Finally, differences in stimulation parameters between the two studies may have influenced the observed connectivity patterns. Healthy participants received a single session of 20-min

stimulation at 4 mA, while patients with DOC underwent stimulation over five sessions on five consecutive days, each lasting 40 min at 8 mA.

In summary, the distinct patterns observed between healthy participants and patients with DOC can be attributed to differences in baseline neural functioning, experimental conditions, connectivity analyses, and stimulation parameters.

5 Limitation and outlook

This dissertation presented a novel rhythmic acoustic-electric stimulation approach and systematically evaluated its effectiveness in modulating consciousness. Despite the encouraging results, there are a few limitations, which need to be addressed in future studies.

Since consciousness is a complex construct, comprehensive assessments require consideration of multiple dimensions. In this dissertation, we reported various indicators used to assess consciousness, including behavioral measures (e.g., the CRS-R scale and the threshold and slope of the psychometric function), event-related potentials (e.g., N400, ARN, and P300), oscillatory brain activity (e.g., gamma and beta brain activity), and network connectivity measures (e.g., PLI and DCM connectivity). We observed the effects of acoustic-electric stimulation on a portion of indicators. However, the relationships between these indicators, as well as their robustness and reliability, remain to be established. Before employing these measures as reliable indicators for assessing treatment effectiveness, further research is needed to determine their reliability and interrelationship. This would involve systematically examining each indicator's sensitivity and specificity in detecting changes in consciousness.

Although we have provided some insights into the potential mechanisms of the stimulation approach, a more comprehensive understanding of the mechanisms underlying the observed effects of acoustic-electric stimulation on consciousness remains to be explored. For instance, investigating neurochemical mechanisms using magnetic resonance spectroscopy (MRS) can contribute to the understanding of the neural transmitter changes associated with the effects of acoustic-electric stimulation, which can be used for guiding the application of acoustic-electric stimulation. Furthermore, we only used EEG in the current studies, which has high time resolution but limited spatial resolution. In future research, using functional magnetic resonance imaging will enable us to investigate the effects of stimulation on more specific brain regions, further enhancing our understanding of the neural mechanisms of its action.

Despite our efforts to optimize the stimulation approach, such as choosing between unimodal or multimodal stimulation, multiple parameters still require further investigation. For example, the optimal number of stimulation sessions and the ideal duration for each session have not been determined. In future studies, a longitudinal approach could be employed to apply stimulation over several weeks while conducting continuous assessments of consciousness. By adopting this design, we can better understand the dose-response relationship and the potential cumulative effects of the stimulation on consciousness.

In our current approach, we assume that synchronizing acoustic and electric stimulation will exert the most significant effect on consciousness. However, as discussed in section 3.2, it remains possible that setting a minor time lag between the acoustic and electric stimulation could have a more substantial effect on STDP, which may affect the offline effects. Future studies should explore the impact of varying time lags between acoustic and electric stimulation on both behavioral and neurophysiological outcomes. By systematically manipulating the time lag and assessing its influence on consciousness and neural entrainment, we can determine the optimal degree of synchronization

between the two modalities. This information will be invaluable for refining the acoustic-electric stimulation approach.

To address brain-behavior causality, we assessed changes in consciousness and gamma brain activity before and after gamma-band stimulation, providing evidence for a causal link between modulated brain activity and observed behavioral changes. Nevertheless, it is crucial to acknowledge that the stimulation approach may not only target cortical oscillations but also other brain activities. It is possible that the modulation of gamma activity merely corresponds with changes of other activities, instead of playing a direct causal role in consciousness. To determine this, future research could focus on selectively manipulating specific brain activity while keeping other activities constant. However, this is currently challenging to achieve and often overlooked in the literature.

Despite these limitations, the current thesis provides significant advances in our understanding of gamma-band acoustic-electric stimulation that can be valuable for further clinical studies. These scientific and potential clinical and societal impacts are described in more detail in Section *Impact paragraph*.

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Appendix

Impact paragraph

Consciousness is everywhere in our daily life, but it remains profoundly mysterious. Research on consciousness is comparatively limited when compared with studies on other cognitive domains, such as memory, attention, and speech. Likewise, the investigation of disorders of consciousness (DOC) has not been as widespread or popular as research on other neurological conditions, such as Alzheimer's disease and Parkinson. Nevertheless, this does not diminish the importance or urgency of investigating consciousness.

Although the prevalence of patients with DOC is difficult to estimate, limited data indicate that approximately 5/100,000 of people are diagnosed with prolonged DOC every year. This DOC state can persist for years or even decades. Notably, patients with DOC are incapable of living independently and require continuous care from families or professional caregivers. The cost of caring for these patients is substantial, causing a considerable burden on healthcare systems.

In my thesis, I developed an innovative stimulation technique at gamma frequency that combines musical stimulation with electrical stimulation on the face. Further inspired by previous research on speech processing, we adopted a speech paradigm and used it for consciousness assessment. We observed that this novel stimulation approach improves consciousness in both healthy individuals and patients with DOC. More specifically, healthy participants demonstrated improved conscious tactile perception and brain responses after the multimodal acoustic-electric stimulation. Patients with DOC showed improved levels of consciousness, as evidenced by behavioral scales, certain aspects of speech processing, and brain oscillations. Furthermore, the improvements in consciousness were accompanied by alterations in brain connectivity.

These findings have both scientific and societal impacts. By investigating the therapeutic potential of acoustic-electric stimulation at gamma frequency on gamma oscillations and consciousness, this thesis provides evidence for the functional role of gamma oscillations on consciousness, and has potential to significantly advance the field of consciousness restoration. By investigating the neurophysiological mechanisms (e.g., functional connectivity) underlying the modulatory effect on consciousness can directly impact neuroscientific theories of consciousness and shed light on potential mechanisms for consciousness restoration. Moreover, the finding that the interplay of acoustic and electric stimulation underlies improvements in consciousness highlights the unique benefits of multisensory integration over unimodal signals, inspiring further exploration and customization of multisensory stimulation in cognitive and translational neuroscience. Furthermore, the promising therapeutic approach of modulating neural oscillations also holds implications for the rehabilitation of other neurological disorders, since a wide range of neurological disorders (e.g., Alzheimer's disease, Parkinson) are characterized by abnormal brain oscillations. This can highly inspire researchers in cognitive and translational neuroscience fields to explore new treatments by targeting abnormal brain responses and develop neuromechanism-driven treatment approaches.

In addition to the scientific impact, this thesis introduces a portable, non-invasive, cost-effective approach that has a high potential to develop into a new treatment for disorders of consciousness. We have conducted a phase 1 study in healthy participants (Chapter 3) and collected class II evidence in a DOC patient study (Chapter 4). In the middle term, when incorporating feedback from patients' families and clinical staff, follow-up phases 2 and 3 multicenter clinical trials should be conducted in collaboration with hospitals and coma centers, aiming to collect further clinical evidence of this novel approach. In the long term, these efforts can help translate the research findings into regular clinical practice and to improve functional recovery and quality of life for patients with DOC. This promising approach can alleviate the burden on families, reduce healthcare costs for society, and provide more options for clinicians.

To disseminate the findings of this thesis and contribute to the scientific exchange and advancement in the field of consciousness, all of these findings have been or will be published in neuroscience or clinical journals to ensure accessibility for both the scientific and clinical communities.

Our research group has proactively shared these findings on social media platforms, such as Twitter, further enhancing the reach and visibility of this work. The research data of Chapters 2 and 4 have been made openly accessible on the Open Science Framework (OSF), and data of other Chapters will be made available upon publication.

In summary, the impact of this Ph.D. thesis is multifaceted, encompassing both scientific and societal aspects, significantly contributing to the fields of neuroscience and clinical care.

Acknowledgement

As I am looking back over these past four years, it is not the tough challenges that stick in my mind. Instead, what stands out is just how lucky I have been. I feel incredibly grateful for having such a solid crowd of people backing me up. Without you all, this Ph.D. journey could have been like sipping on plain water – lacking any special taste. But you have acted as the perfect blend of spices – sometimes acting as the salt that provided a unique perspective, sometimes acting as the sugar that brought in a touch of optimism – you have truly enriched my Ph.D. experience. So, here is a heartfelt thanks to all of you.

My deepest gratitude goes to my supervisor, Lars. I realize that expressing how great a supervisor you are and all the gratitude I have for you could easily fill up two pages. But I would attempt to keep it concise here (though it is just a tiny fraction of how great you truly are). During our very first Skype interview on Nov 30, 2018, when I shared my research interests outside the field of auditory perception, you showed your great patience and support. You provided detailed and constructive feedback on my research proposal for many rounds, always valuing my ideas even though many of them were naive. During the first few months in Maastricht, our biweekly meetings were super helpful in guiding me through this transition period. After that period, our weekly meetings continued to be a steadfast source of support throughout my entire four-year Ph.D. journey. I could always count on you to provide solutions to my dilemmas and relieve my stress after our discussions. Our brainstorming sessions during these meetings were undoubtedly the highlights of each week.

Your efficient and smart problem-solving skills, combined with your creative ideas, have made a significant impact on my academic growth. You are always open to suggestions and opinions, while maintaining a unique perspective when dealing with “authority” (e.g., some reviewers). You can always balance between being receptive and standing firm on your own viewpoints. Though I am not as brave as you, I am learning and growing 😊. I also appreciate your great patience with me. I still vividly recall the meetings when you invested nearly six hours guiding me through the structure and writing process of my first paper. It is the best writing lesson I have ever had.

What truly moved and motivated me was your unwavering trust in me and encouragement, especially during tough times like paper rejections and doubting about my value in the job market. Your assistance extended beyond academics, helping me with many other things such as visa applications and finding a nice bicycle. Working with you has made my Ph.D. journey a time of not only intense learning but also personal growth and enjoyment. I believe our collaboration will continue even after my graduation. Thank you, Lars.

Bettina, I still remember the first time we met. You advised me to always lock the door and bring my keys when leave the office. Though it seemed like a small tip at the time, it turned out to be a crucial piece of advice after a few occasions of locking my keys inside. This attention to detail is also how you approach research, always pointing out that easy-to-miss but crucial information. Whenever your office door was open and I happened to pass by, I would often hear my name, “Min”, which always led to warm greetings and nice conversations. Our many spontaneous chats in the corridor, your office or even at the bicycle parking area have always been cherished. The experiences and advice you have shared with me, like bouncing back from paper rejection, not being overly concerned with the Impact Factor, tips on grant applications, and deciding whether to stay in academia, have guided me through many significant moments of my Ph.D. I am also grateful for you sharing your successful Rubicon-grant application form with me, which gave me an easier start for the application. I enjoyed the dinner in your garden in August 2022. The combination of German beer, delicious food, and great conversation made for a memorable evening and remains one of the best experiences of my Ph.D.

Elia, I have greatly appreciated your intellectual input during our section meetings. Your profound knowledge, critical thinking, and refined taste for research have been a source of inspiration. I have often regarded your feedback as an early test for my work before convincing reviewers. I enjoyed our conversations during the auditory conference in Magdeburg in 2022. You are always

happy to share your insights and to give help. I am immensely grateful for the recommendation letter for my postdoctoral grant application, which undoubtedly played a crucial role in the positive outcome. Thank you, Elia, for your support and guidance.

I would like to express my great appreciation to my thesis committee, thank you for taking the time to thoroughly review my thesis. I would also like to express my special appreciation to the China Scholarship Council (CSC 201906320078) for their support, which made my studies abroad possible.

I would also like to extend my thanks to all the members of the auditory group. Lars H, Federico and Fren, thank you for providing valuable suggestions on my projects and providing detailed explanations when I have questions. Giancarlo, thank you for sharing the Matlab book and answering my statistic questions. Mahdi, I enjoyed the dinners and talks with you. Michele, thank you for being around at the ICAC conference. Lonike, I was charmed by your smile when we first met, and I am grateful for your effort in organizing many MBIC activities. Michelle, Isma, Agustin, Miriam, Maria, and Gijs, thank you for fostering such a pleasant environment in the auditory group.

As for my colleagues at CN, there are too many names to list. There have been many memorable moments with you all, such as the CN Christmas party, CN lunch, and retreats. I also thank our secretarial team: Christl, Eva, and Andra. Your support has been indispensable and has greatly eased my path.

Prof. Luo, deciding to join your group as a master's student has been one of the best decisions I have ever made. Your extensive clinical and research experience, combined with your unique and intuitive understanding of diseases, continues to inspire me — it is truly an extraordinary talent. Even though I was not a direct member of your group following my master's graduation, you have consistently provided immense support for my projects. When I was stressed and anxious a few months before graduation, you tried your utmost (e.g., keeping your lab open for me and always being willing to be my referee) to ease my anxiety. Furthermore, thank you for having always been open to offer me a position in your lab. However, when I got a grant, you respected my decision to go abroad and expressed genuine happiness about my new opportunities. I have always felt so lucky and safe knowing you have got my back.

You have also treated me like family, checking in on me nearly every week, celebrating each important moment in my life, offering unwavering support during my challenging times, and inviting me for dinner during my holidays in China. You even helped care for Sheng when I was abroad, making him less lonely. You are always happy to share your experiences with me. It is never felt like a lesson, but rather, like a relaxed conversation between family members. It is really surprising to see how similar our personalities are, which has kept us closely connected despite the physical distance and even brought us closer than we were four years ago.

Dr. Nai Ding, you are the type of researcher I aspire to be — brilliant, innovative, and precise. For you, research is like creating art, using your own palette to decorate science. I am grateful for your recommendation to join the auditory group in Maastricht, which was the first step in my Ph.D. journey. Your guidance and advice over the years about my career and research have been invaluable. I am enthusiastic about our ongoing collaborations, and I hope I can get fewer comments: “too low” 😊.

Dr. Fali Li, you have not only been a great collaborator and unofficial supervisor but also a cherished friend. Your unwavering encouragement, and at times, the positive pressure, have continually motivated me. I am confident that your efforts will be richly rewarded, and I am looking forward to our future collaborations. I also wish to extend my thanks to Dr. Jian Gao for his invaluable assistance in my research and for addressing countless clinical questions. Lastly, my appreciation goes to all participants, both the students at UM and the DOC patients, along with their families in China. Your trust and willingness to contribute data have been fundamental to my research.

Manli, you are a person who I truly believe deserves all wonderful things. Your brilliance and unwavering efforts on your own path have made you an excellent researcher. Your integrity, resilience, and reliability make you a cherished friend. During my first month in Maastricht, your warm welcome made me feel at home. Throughout the initial three years of my Ph.D., you were the person I instinctively turned to for advice. Conversations with you always provided me with direction, whether the matter at hand was related to science or life in general. In my final year, during times of dilemma, I often found myself wondering what advice Manli would offer. This tells how significant your impact has been on me and how much I miss you. I wish you all the best in your job hunting, and I hope you can always pursue what you like, and that the things you decide to do are what you like. I will continually take pride in every step you take on your journey.

Maite, I was wondering why we did cross paths so late. Even though we have shared countless coffee breaks, they never seem to be enough. When we look at our to-do lists, there are always endless things waiting for our attention; it seems like we are racing against time. However, I hope our list remains long and growing, regardless of whether I move to China or the UK. This way, we will always have a reason to meet and things to look forward to. Our daily conversations are a highlight of my day and a beacon during gloomy ones. As someone who often hesitates and gets emotional, you push me forward and provide me with a different perspective. That makes my life easier and makes me brave. Thank you! Now I am looking forward to our future travels to Japan and China. I wish you and Glenn good luck in your final year of Ph.D.

Ting, we will always remember the first and last few months of our Ph.D. journey. It was a truly magical time. We were living in our small Ph.D. group (just the two of us), and even though it was naive, it was also cozy. It was so lucky to have you around during my Ph.D. You are caring, kind, and smart, so I believe you will have a fulfilling future ahead.

Chen, you attracted me from our first talk. I agree that individuals with similar personalities naturally attract each other and that is certainly true for us. Despite not having many conversations, I place absolute trust in you and always feel happy when I stop by your office to exchange thoughts. Your optimistic attitude towards life, coupled with your distinct care for others, truly influences and attracts friends around you.

Ema, you are a really fascinating person. You have a clear understanding of what you want and are not influenced by conventional thinking. This makes both life and research seem less difficult for you, and I trust that you can handle anything that comes your way in the future. I deeply miss those “girls' nights”. And thank you for preparing such wonderful Croatian food. Our 5-hour chat during the Eurovision final night was genuinely enjoyable, and your unique perspective always adds an interesting layer to our discussions. I look forward to more “girls' nights” in the UK.

Lidongsheng, your sincere personality has always been appealing. Thank you for checking in on me when I was mentally disconnected and for understanding my prolonged silence. I appreciate your willingness to lend an ear whenever I have needed to talk and for offering your unique perspectives. I am also grateful for the trust you have placed in me, sharing your uncertainties/dilemmas and valuing my suggestions. I wish you all the best in your ongoing projects and future career. I wish for Diandian's continued good health and that she will be there to share in many more of your significant milestones. I look forward to the day when our paths may cross again.

Xueying, I will forever cherish the memories we made during the curfew period and the numerous travels we had together. You are always a reliable source for the most up-to-date information, both in research and life. I admire your ability to search for the latest information and your consistent willingness to help others. Your help has genuinely made my life easier.

Juanzhi, I am delighted that our paths crossed again in Maastricht and you have brought so much fun into my life. It is because of you that I have fallen in love with Hunan cuisine, and you have

significantly increased my tolerance for spicy food. I am grateful for your encouragement, the numerous hotpot dinners we have enjoyed, and many nights filled with drinks and games.

I would also like to thank my friends in China. Cai, thank you for your constant care and for always managing to calm me down during my moments of struggle. Lao Yueqiong, I greatly admire your ambition and your unwavering commitment to work diligently towards your goals. I hope that your dreams will come true. Despite the annoying Covid, it was great to meet both of you in June 2023. To Qian Chunjie and Zeng Yinshuang, who have been my friends for twenty years, thank you for your constant support. To my high-school couple friends, I am overjoyed to hear about your new baby, and I wish you happiness in your life together. Bao, thank you for always being there and for sharing your life with me. Please remember this: you are far more excellent than you think, so please be confident and be brave to chase your dreams.

I would like to thank my office mates. It is super lucky to have shared the office with both of you. Johannes, you are a really warm person and have a special sense of humor. I am surprised that you are good at all games and I wonder if there is anything you can't excel at. Sven, even though you are not a social person, I have found you to be warm and reliable, always willing to lend a hand. I greatly appreciate your patience in answering all my questions regarding grants, salary, and graduation matters. I wish you both the best in the future.

My special thanks go to Sheng. Thank you for always making me your top priority and for moving to Maastricht just because of ME. Thank you for giving me your great patience, unwavering support, utmost care, and immeasurable love. You are the one with whom I can instantly share both my moments of joy and hardship. Without your understanding and encouragement, I could not have accomplished many things. Thank you for always being my greatest supporter, my biggest fan, my best friend and my soulmate. I am looking forward to continuing our exploration of this wonderful world, always hand-in-hand.

感谢远在国内的爸爸妈妈。过去四年, 为了照顾我的时差, 你们总在国内时间深夜或凌晨和我视频, 通过屏幕与我分享生活点滴。尽管心中满是想念, 你们尽力掩饰, 只为让我少一份牵挂, 更专心地做我的事。离家越远, 思念越浓烈, 只愿你们的碎碎念念可以伴随我的岁岁年年。大伯, 从小到大的家长会一直都是你出席。一路陪伴, 一路指引, 使我成为一个怀揣梦想, 心中有爱的人。阿姨, 谢谢你把天真善良的儿子交给我, 成为我的最佳人生伴侣。你如月光, 静静守候; 我们深知在杭州一直有个温馨整洁的家, 不论何时。外婆, 总是惊叹您的智慧。与您的一通电话总能让我茅塞顿开。衷心祝愿您健康、长寿, 愿我们多些时光, 伴您左右。

Curriculum vitae

Min Wu was born on August 21, 1993 in Zhejiang, China. She completed her secondary education at Longyou Middle School in 2011. Subsequently, she pursued higher education at Guangzhou Medical University, where she received her Bachelor's Degree in Clinical Medicine in 2016. She then embarked on a Master's program in Neurology at Zhejiang University under the supervisor of Prof. Benyan Luo. From 2016 to 2019, Min has completed her clinical rotations and obtained the certificate as a clinical doctor. In 2019, she obtained her Master's degree. Her thesis focused on the diagnosis and prognosis of patients with disorders of consciousness. Later that year, she was awarded the China Scholarship Council (CSC) scholarship, which enabled her to continue her academic journey as a PhD student in Cognitive Neuroscience at Maastricht University. Min worked with Dr. Lars Riecke, Dr. Bettina Sorger and Prof. Elia Formisano on projects to modulate consciousness with acoustic-electric stimulation in both healthy individuals and patients with disorders of consciousness. In July 2023, Min was awarded the Rubicon grant from NWO to support her postdoctoral research abroad. Starting in October 2023, Min will work as a postdoctoral fellow at the University of Oxford, where she will focus her research on treatments for stroke patients.

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Manuscript submitted

Wu, M., Auksztulewicz, R., Riecke, L. (Under review). Multimodal acoustic-electric trigeminal nerve stimulation modulates conscious perception.

Conference Contributions

Wu, M., Riecke, L. (2022). Oscillatory auditory-tactile stimulation modulates perception. *International Conference on Auditory Cortex (ICAC), Magdeburg, Germany*

Wu, M., Bosker, H.R., Riecke, L. (2022). Dynamics of word processing during speech parsing. *Speech in noise workshop (SPIN), online*

Wu, M., Bosker, H.R., Riecke, L. (2021). Dynamics of auditory word processing during speech parsing. *WASdag: Meeting Werkgemeenschap Auditief Systeem, Maastricht, the Netherlands, online*

Wu, M., Bosker, H.R., Riecke, L. (2020). Sentence structure effects on auditory word processing. *Advances and Perspectives in Auditory Neuroscience (APAN), online*

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