Research article

Title page

Title

Phase-synchronized 40 Hz transcranial electric and magnetic stimulation boosts gamma oscillations and working memory

Abbreviated title

40 Hz tACS and iTBS and gamma oscillations

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Abstract

Gamma oscillations play a crucial role in core cognitive functions such as memory processes. Enhancing gamma oscillatory activity, which is reduced in Alzheimer's Disease (AD), may have therapeutic potential, but effective interventions remain to be determined. This study applies novel noninvasive brain stimulation techniques, namely phase-locked 40-Hz intermittent theta-burst stimulation (iTBS) and transcranial alternating current stimulation (tACS), and explores gamma oscillations and working memory changes. In 300 experimental sessions conducted on 30 participants, the effects of 40-Hz tACS, 40-Hz iTBS, two combined interventions (phase-locked iTBS to tACS peak sine wave or tACS trough sine wave), and a sham condition were explored. Gamma oscillatory activity (for 2 hours after intervention), working memory (3-back and 1-back load), and brain functional connectivity were monitored following each intervention. All stimulation protocols enhanced 40-Hz oscillatory power, with the iTBS-tACS Peak showing the most significant and stable increase, followed by 40-Hz tACS and 40-Hz iTBS. These stimulation protocols improved functional connectivity during the 30 minutes post-intervention while participants performed a memory task. Only the 40-Hz tACS and iTBS protocols enhanced high-load working memory speed. Concurrent, peak-synchronized 40 Hz iTBS combined with tACS and 40-Hz tACS may be a reliable protocol to induce long-lasting oscillatory activity in the gamma frequency range. These protocols may have therapeutic effects in patients with Alzheimer's disease.

Keywords: Gamma oscillations; Memory; Alzheimer's disease; Transcranial alternating current stimulation; theta burst stimulation; Prefrontal cortex.

1- Background

The prevalence of Alzheimer's disease (AD) is expected to rise threefold by 2050 making AD a serious global health threat [1]. Recent forecasts estimate the global direct costs of AD for the public health system to increase from approximately 2.8 trillion US dollars (USD) in 2019 to 16.9 trillion USD by 2050 [2]. Besides this current trend, treatment plans for patients mostly focus on symptom management [3]. In addition, new therapeutics have been introduced to reduce accumulated amyloid beta (Aβ) plaques, one underlying pathophysiological mechanism of disease progression, and show modest clinical benefits, but can lead to adverse events such as brain bleeding and edema [4]. Thus, treatment options for AD are currently limited.

A recent treatment approach involves non-invasive brain stimulation (NIBS). The most prominent categories of NIBS are transcranial magnetic stimulation (TMS) and transcranial electrical stimulation (tES). In TMS, the brain is stimulated by applying single or repetitive magnetic pulses at a specific frequency to the skull. Through electromagnetic induction, magnetic pulses induce an electrical current flow in the underlying brain tissue that lead to changes in electrical and biochemical brain activity [5]. In particular, the repeated application of pulses, repetitive transcranial magnetic stimulation (rTMS), is widely used in neurology, psychiatry, and basic neuroscience to induce long-term potentiation (LTP) and long-term depression (LTD) -like plasticity effects [6] and induce short-lasting changes in oscillatory neural activity in the cerebral cortex [7–9].

Similar physiological effects are observed for tES interventions, mostly in transcranial direct current stimulation which can induce LTP- and LTD-like plasticity comparable to rTMS. In addition, electrical stimulation with an alternating current, transcranial alternating current stimulation (tACS), interacts with endogenous oscillatory activity by aligning ongoing oscillatory brain activity to the weak exogenous current produced by tACS [10]. This mechanism is called entrainment. The entrainment effects of tACS outlast the acute stimulation phase, leading to longer-lasting changes in oscillatory activity than the short-lasting modulatory effect on oscillations produced by rTMS [11].

Recent evidence suggests that Aβ plaques disrupt plasticity mechanisms and are associated to abnormal oscillatory brain activity in patients compared with healthy controls [12,13]. Both processes can be modulated using NIBS interventions. Animal and human studies have suggested gamma oscillations (30–100 Hz) as a promising target frequency for the treatment of patients with AD. Previous investigations have shown decreased power of gamma oscillations in the electroencephalogram (EEG) of AD patients [14] and in intracranial recordings of animal models [15,16]. Gamma oscillations play a pivotal role in working memory and the formation and retention of declarative memory [17–19]. Moreover, studies in AD animal models observed increased Aβ clearance post-intervention [20,21]. The modulation of 40Hz oscillations has been investigated in basic, preclinical, and clinical trials, showing increased Aβ clearance in patients and animal models and improved cognitive functioning in AD patients [15,22]. Modulation of 40Hz oscillations improves working memory in clinical populations, whereas evidence for the effectiveness of 40Hz neuromodulation regarding working memory improvements in healthy samples is limited [23,24].

NIBS techniques aiming to interact with 40Hz oscillatory activity could be a promising approach for AD treatment and optimizing available protocols is expected to lead to improved outcomes. A recent research approach focusing on the combination of different NIBS techniques showed that the combination of rTMS applied to the peak of the alternating current produced by tACS led to a stronger and longer-lasting increase of mid-range oscillatory power (theta and alpha EEG bands) [25–27] while for the low-frequency band (i.e., delta) rTMS applied to the trough of the alternating current was most effective [28]. To our knowledge, no study investigated the effects of this combined stimulation approach for the gamma band. The present study investigated the impact of five distinct non-invasive brain stimulation (NIBS) protocols on 40Hz oscillatory power and their influence on working memory performance in healthy adults. We hypothesized that applying intermittent theta burst stimulation (iTBS), a high-frequency rTMS protocol, at the peak of the sinusoidal waveform generated by tACS would produce greater and more sustained increases in

40Hz oscillatory activity and enhanced working memory performance compared to iTBS applied at the trough, tACS alone, iTBS alone, or sham stimulation [26,27].

2- Materials and Methods

2.1. ***Participants***

Thirty healthy subjects (20 females, mean age = 26.54, SD = 3.74) were recruited from the Technical University Dortmund, Ruhr-University Bochum, and the surrounding community. All subjects had normal or corrected to normal eye vision, were right-handed, non-smokers, and had no history of neurological or psychiatric disorders including seizures or epilepsy, CNS medication intake, metal implants, and current pregnancy. To further verify the health status of the subjects, an examination was carried out by a medical doctor before the start of the experiment. Written informed consent was signed by all subjects. The study conformed with the latest version of the Declaration of Helsinki and was approved by the ethics committee of the Leibniz Research Centre for Working Environment and Human Factors (ID: IfADo-196). Participants were monetarily reimbursed and allowed to withdraw from the study at any time.

2.2. ***Intervention***

Five interventions with different combinations of iTBS and tACS were tested: (1) *X-tACS Peak* protocol in which the applied iTBS pulses were synchronized to the peak of the target site's tACS sinusoidal waveform (90° phase from wave onset), (2) *X-tACS Trough* protocol in which these iTBS pulses were synchronized to the trough of the tACS-generated sinusoidal waveform (270° phase from wave onset), (3) the intermittent 40Hz theta burst protocol (iTBS alone) with sham tACS, (4) a 40Hz tACS protocol (tACS alone) with sham iTBS, (5) and a sham protocol (see Figure 1b-e.).

We used a modified iTBS protocol (intra-burst interval of 40 Hz instead of 50 Hz), applied at 80% of the individual subjects' active motor threshold (AMT) four times within a 20-minute time frame starting at 0 minutes, 5 minutes, 10 minutes, and 15 minutes from the beginning of the

intervention resulting in 2400 delivered pulses for the whole intervention at each stimulation side. The tACS was delivered utilizing a Starstim 8-channel constant current, battery-powered electric stimulator (Neuroelectrics, Barcelona, Spain). Circular carbon rubber electrodes (2cm radius, 12,57 cm^2) were used for stimulation. During tACS, current was delivered with a 1mA peak-to-peak amplitude with a 15-second ramp up at the beginning of the stimulation and a 15-second ramp down at the end of the stimulation. Sham tACS was applied by only ramping the current for 15 seconds up and 15 seconds down at the beginning and end of the stimulation respectively. A customized circuit was developed in-house to combine tACS and the modified iTBS protocol by phase-locking iTBS pulses to the tACS waveform. iTBS pulses were delivered using two figure-of-eight-shaped TMS coils. For sham iTBS, two different sham TMS coils (double 70mm pCool-SHAM coil, Mag&More GmbH, Munich, Germany) generated audible clicks at the same intensity as an active coil without inducing an electromagnetic pulse. To minimize the delay between iTBS pulses from the two coils, the TMS devices were connected in series, with TTL signals used for triggering. An oscilloscope confirmed near-zero latency between the pulses. Details of the intervention are in the supplementary material.

*2.3. **Neuronavigation***

To control for movement of the TMS coils during the stimulation, an MR-based 3D-navigation system (LOCALITE GmbH, Germany) was utilized to monitor the orientation and position of the TMS coil to the 3D-head model and fiducials (nasion, left and right ear) of the participants derived from individual anatomical MRI scans. The T1 weighted structural scans were acquired by a Siemens 3T Prisma scanner based on the 3D-MP-RAGE sequence (TR=2600 ms, TE=2.92 ms, flip angle=12°, FoV read=256 mm, FoVphase=87.5%, 176 slices with $1\times1\times1$ mm^3 resolution).

2.4. ***Measures***

2.4.1. Electroencephalography

EEG was recorded using a 64-channel EEG System (Bittium, NeurOne, Bittium Corporation, Finland) in a shielded room with stable light conditions. The Ag/AgCl EEG electrodes were attached to the head using high-viscosity electrolyte gel (SuperVisc, Easycap, Herrsching, Germany). All recordings were sampled with a sampling rate of 2000 Hz. The online reference electrode was placed at FCz and the ground electrode was placed at CPz. The EEG impedance was kept below 5kOhms throughout the whole recording. Digital TTL triggers were transmitted to the EEG system by a server PC to indicate the start of a condition (eyes open or eyes closed) during the resting state recordings. For EEG preprocessing, BrainVision Analyzer version 2.2.2 (Brain Products GmbH, Gilching, Germany) was used. The pipeline was adapted from Salehinejad et al. (2021). EEG recordings were bandpass-filtered between 0.1 and 90Hz (zero phase shift Butterworth filters) with a notch filter at 50Hz to remove line noise. All electrodes were referenced to a common average reference montage. The vEOG signal from channel Fp2 was used for the correction of ocular artifacts using the Gratton and Coles method [30] in line with our previous works [29-30]. Channels with artefactual signals were identified and spline-interpolated. Resting state EEG recordings were then segmented into two-second epochs, artifactual epochs containing EEG activity greater than a 100mV peak-to-peak amplitude automatically marked, visually inspected, and removed manually. Artifact-free segments were then transformed from the time to the frequency domain using Fast Fourier transform (Hanning window length 10%, zero padded) and averaged.

2.4.2. N-Back working memory task

The effects of the stimulation protocols on working memory were assessed with a computerized N-Back working memory task following our previous work [27] using the E-Prime 3.0 software (Psychology Software Tools, Pittsburgh, PA) (For details see supplementary materials).

2.5. ***Experimental design and procedure***

Participants attended 10 experimental sessions (see Figure 1a), all scheduled at the same time of the day (9 am or 1 pm) to control for arousal levels. Sessions were spaced at least seven days apart to prevent carry-over effects. EEG experiments were conducted in a shielded, soundproof chamber with consistent lighting. Upon arrival, participants were seated in a reclined chair, and a 64-channel EEG cap was fitted according to the 10-10 international electrode placement convention. tACS electrodes were pre-placed over the target regions (F3, F4, TP9, and TP10). After EEG setup, a vacuum pillow was used to stabilize the head position. The TMS intensity for iTBS was determined in each session separately to account for changes of cortical excitability. Baseline EEG recordings consisted of two minutes each of eyes open (EO) and eyes closed (EC) conditions. During the EO recording, participants fixated on a black cross against a white background at one meter eye distance.

In the behavioral working memory sessions, participants completed six blocks of the N-Back task (three 1-Back and three 3-Back blocks in alternating order), with block order counterbalanced across participants but consistent within sessions. Each block featured a new random sequence of letters, and participants trained on the 1- and 3-Back conditions before the first session, with training deemed successful if accuracy exceeded 70%. After baseline recordings, TMS coils were positioned over the F3 and F4 electrode sites at a 45° angle to the midline, with the handle pointing backward. Participants then received 20 minutes of stimulation with different protocols in randomized order across sessions. Following stimulation, two minutes of EO and two minutes of EC resting state EEG recordings were collected (T1), and this process was repeated every ten minutes up to 120 minutes post-intervention (T13). In the N-Back sessions, participants performed six blocks of the N-Back task immediately after the intervention.

Insert Figure 1 here

2.6. ***Statistical analysis***

Data were analyzed using R version 4.4.0 (R Core Team, 2024) and GraphPad Prism version 9.5.1 (GraphPad Software, Boston, Massachusetts USA). Physiological data (EEG power spectrum and functional connectivity) were preprocessed and analyzed with Brainvision Analyzer, Fieldtrip, and Brainstorm toolbox [31,32]. Figures were created using Prism 9.5.1, Microsoft PowerPoint, and MATLAB 2023b utilizing the Fieldtrip toolbox. Details of data analysis (physiology, behavior, blinding, and side effects) are presented in the supplementary material.

3- Results

3.1. Data overview, blinding, and side effects

No significant differences were observed in participant ratings whether real (X-tACS Peak, X-tACS Trough, tACS, iTBS) or sham stimulation was applied, confirming the effectiveness of blinding (Table S1).

The side effects reported immediately and 24 hours post-stimulation are summarized in Tables S2 and S3. No significant differences were observed in tingling, burning, pain, or skin redness across stimulation conditions. However, a significant effect of stimulation on visual phenomena was detected during the five behavioral sessions (Table S4). Post hoc analyses revealed significantly increased visual phenomena for tACS ($t = 3.40$, $p = 0.002$), X-tACS Trough ($t = 3.78$, $p < 0.001$), and X-tACS Peak ($t = 3.51$, $p = 0.001$) compared to sham, but not for iTBS ($p = 0.643$). Similarly, during the five resting EEG sessions, significantly larger visual phenomena were observed for tACS ($t = 3.67$, $p = 0.001$), X-tACS Trough ($t = 3.54$, $p = 0.001$), and X-tACS Peak ($t = 3.61$, $p = 0.001$), while iTBS again showed no significant difference ($p = 0.801$).

Follow-up correlational analyses indicated a significant positive correlation ($p = 0.02$) with moderate strength ($r = 0.45$; see Figure S2) between visual phenomena and post-intervention 40Hz oscillatory power in the frontal ROI (EEG sensors: F3, F4, Fz, F1, F2, AF3, AF4, FC3, FC4, F5, F6) specifically for the tACS protocol in the EO condition.

3.2. Stimulation effects on 40Hz oscillatory power

The linear mixed model (LMM) analysis revealed no significant baseline differences between the absolute 40Hz power of the different stimulation protocols in the EO and EC conditions in the frontal ROI and at the whole brain level (Figure S3). Temporal dynamics of the interventions focusing on the frontal region of interest were analyzed using separate LMMs for the EO and EC recording conditions. The analyses revealed significant main effects of stimulation (EO: $F_{4,6979}$= 43.90, $p<0.001$; EC: $F_{4,7111}$=17.25, $p<0.001$), time (EO: $F_{4,6978}$= 85.41, $p<0.001$; EC: $F_{4,7111}$=121.82, $p<0.001$), and electrode (EO: $F_{10,6978}$=5.36, $p<0.001$; EC: $F_{10,7111}$=4.01, $p<0.001$), and a significant stimulation × time interaction (EO: $F_{16,6978}$= 5.86, $p<0.001$; EC $F_{16,7111}$=2.03, $p<0.001$) (Table 1). Intervention effects were observed in eyes-open and eyes-closed conditions. Subsequent pairwise comparisons revealed temporal and stimulation-specific effects.

3.2.1. 40Hz oscillatory power at frontal ROI

3.2.1.1. Eyes open condition

Compared to baseline, X-tACS Peak, tACS, and iTBS significantly increased 40Hz power during the first 30 minutes post-intervention, (all $p<0.001$), with no effect for X-tACS Trough, and sham conditions. At the 30-60 minutes time window, all active protocols increased 40Hz power (all $p<0.001$) and remained significant until 120 minutes post-intervention (all $p< 0.001$). Sham stimulation did not produce significant changes besides the 90-120 minutes time window when an increased 40Hz power was observed ($t=3.08$; $p=0.010$; Table S8). When compared to the sham condition, X-tACS Peak, X-tACS Trough, tACS, and iTBS protocols significantly increased 40Hz oscillatory power during the first 30-minutes post-intervention (all $p<0.05$). The effects of X-tACS Peak, tACS and iTBS stimulation were not statistically different from each other. Furthermore, X-tACS Peak, tACS and iTBS stimulation protocols had a stronger increase of 40Hz gamma oscillations compared to the X-tACS Trough protocol (all $p<0.05$).

In the 30-60 minutes time window, all protocols except tACS resulted in a significant increase in 40Hz oscillatory power compared to the sham condition (all $p < 0.01$). Here, the X-tACS Peak protocol produced the most substantial enhancement, significantly surpassing the effects produced by X-tACS Trough, tACS, and iTBS (all $p<0.05$). No other significant effects were found. In the 60-90 minutes time window, all protocols significantly increased 40Hz oscillatory power compared to sham (all $p<0.001$). X-tACS Peak and tACS showed the largest effects, both outperforming iTBS and X-tACS Trough ($p<0.001$). Finally, during the 90-120 minutes time window, all protocols maintained significantly higher 40Hz oscillatory power compared to sham (all $p<0.001$). The X-tACS Peak stimulation condition induced a significantly larger increase than both X-tACS Trough and iTBS (all $p< 0.05$), but not compared to tACS ($t = -1.99$, $p=0.07$). Differences between iTBS, X-tACS Trough, and tACS conditions were not statistically significant (Figure 2a.; for detailed statistics see Table S7/8 for effects in other frequency bands see Figure S4).

3.2.1.2. Eyes closed condition

For a summary of effects in the EC condition please refer to Figure 2b. A detailed description of the effects is presented in the supplementary materials.

3.3. 40Hz oscillatory power at whole-brain level

The effects of stimulation on the whole brain level were investigated using separate LMMs for EO and EC recording conditions. Significant main effects were found for stimulation (EO: $F_{4,38638}=236.19$, $p<0.001$; EC: $F_{4,38309} = 149.64$, $p<0.001$), time (EO: $F_{4,38637}=470.71$, $p<0.001$; EC: $F_{4,38309}=737.70$, $p<0.001$), electrode (EO: $F_{60,38637}=8.94$, $p<0.001$; EC: $F_{60,38309}=7.01$, $p<0.001$), and significant interactions for stimulation × time (EO: $F_{4,38637}=29.10$, $p<0.001$; EC: $F_{4,38309}=17.01$, $p<0.001$) and electrode × stimulation (EO: $F_{240,38637}=2.19$, $p<0.001$; EC: $F_{240,38309}=2.22$, $p<0.001$). No other significant effects were found (Table 2).

3.3.1. Eyes open condition

Compared to the baseline, the X-tACS Peak protocol significantly increased 40Hz oscillatory power in the frontal and temporal regions of both hemispheres within the 0-30 minute window. The tACS protocol enhanced 40Hz power in the frontal, parietal, and occipital regions, while the iTBS protocol increased 40Hz oscillatory power in the right frontal and right parietal cortices. No significant effects were observed for the X-tACS Trough and sham stimulation protocols. In the 30-60 minutes time window, the X-tACS Peak and X-tACS Trough protocol significantly enhanced 40Hz oscillations, particularly in the frontal and temporal regions. The tACS protocol continued to demonstrate a significant increase in the target frequency power, particularly in the frontal and occipital regions. No significant changes were observed for the iTBS and sham stimulation protocols during this period.

During the 60-90 minutes interval, the X-tACS Peak protocol significantly increased 40Hz oscillatory power across the frontal, temporal, parietal, and occipital cortices. The significant effects from the previous time window for the X-tACS Trough intervention continued. The tACS intervention exhibited intensified effects, in 40Hz power across the same regions. For the iTBS protocol, a significant increase in 40Hz power was observed around the stimulation sides, extending to the right parietal cortex. No significant effects were noted for sham stimulation. In the 90-120 minutes post-intervention, significant effects from the X-tACS Peak, X-tACS Trough, and tACS protocols were maintained. The X-tACS Trough protocol also showed an additional significant increase in 40Hz oscillatory power in the left parietal cortex. The effects of the iTBS protocol intensified, revealing new significant increases in the occipital cortex, while sham stimulation showed no significant effects (Figure 2c).

Compared to the sham condition, the X-tACS Peak protocol enhanced 40Hz oscillatory power in the frontal and temporal cortices of both hemispheres within 0-30 minutes post-intervention while the iTBS protocol effects on 40Hz oscillatory power included frontal and parietal cortices. For

tACS, a significant 40Hz power increase was observed in only two frontal electrodes, while the X-tACS Trough protocol showed a significant decrease of respective oscillations in one right parietal electrode. The effects from the X-tACS Peak protocol were sustained in the 30-60 minute window, and a significant increase in 40Hz power was noted for the X-tACS Trough protocol in the frontal cortex, with no significant effects for tACS and iTBS. In the 60-90 minute period, the significant effects of the X-tACS Peak protocol intensified, with additional increases in the bihemispheric parietal and occipital cortices. The X-tACS Trough effects were sustained, and tACS also showed a significant increase in 40Hz power across the frontal, temporal, parietal, and occipital cortices. The iTBS protocol revealed a focal significant increase in oscillatory power around the stimulation sites and in the right parietal cortex. In the 90-120 minute window, all active stimulation protocols maintained and intensified their effects, with an additional significant increase in 40Hz power in the left parietal cortex for the X-tACS Trough protocol (Figure 2e).

3.3.2. Eyes closed condition

For a summary of effects in the EC condition please refer to Figure 2d/f. A detailed description of the effects is presented in the supplementary materials.

Insert Figure 2 here

3.4. Behavioral performance

3.4.1. Performance accuracy

The results of repeated measures ANOVAs conducted on accuracy and *d* prime (sensitivity index) variables showed no significant main effects of stimulation, time, and their interaction on working memory performance accuracy and the *d* prime index, which shows how well someone can differentiate between "signal" (the target) and "noise" (the distractors) (see Table S7 and Figure 3e-f). However, for performance speed, the results of the repeated measures ANOVAs conducted on

absolute and standardized RT of 3-back trials showed a significant main effect of time on both absolute RT ($F_{1,92507}$= 22.02, p<0.001) and standardized RT ($F_{1,0.189}$= 14.56, p<0.001) but no significant main effect of stimulation or time×stimulation interaction (Table S11). FDR-corrected post hoc t-tests on absolute RT (pre vs post-intervention) for each stimulation condition indicated no significant improvement in response speed following each stimulation protocol compared to baseline performance (Figure 3a). However, response speed improved trendwise following the iTBS (-0.68.22 ms) and tACS (-0.56.79 ms) interventions.

Subsequent FDR-corrected post hoc t-tests were carried out on the RT difference score (post-stimulation RT – pre-intervention RT), revealing a significantly larger difference for iTBS compared to the sham condition (Figure 3b). Between-group pairwise comparisons showed no differences between the sham and other active conditions in pre-intervention and post-intervention values. For standardized RT, however, FDR-corrected post hoc t-tests showed a significantly faster response after iTBS (t= 3.24, p=0.006) and tACS (t= 2.74, p=0.032) conditions as compared to the baseline (pre-intervention performance), and response speed was significantly faster in both interventions as compared to the sham condition too (t_{iTBS}= 3.30, p=0.004; t_{tACS}= 3.30, p=0.021; Figure 3c). For reaction time variability, FDR-corrected post hoc t-tests showed no significant difference in performance speed variability after each stimulation condition compared to baseline performance (Figure 3d). No differences were found between the sham condition and the active conditions in pre-intervention and post-intervention values. No significant effect of stimulation, time, or their interaction was found on 1-back load (see Table S11 and Figure S5).

Insert Figure 3 here

3.5. Stimulation effects on functional connectivity: Global Efficiency

We conducted an a priori whole-brain functional connectivity analysis to see how the respective interventions affect functional connectivity. We specifically analyzed global efficiency in

the first 30 minutes post-stimulation (during which the task was conducted). This metric has been demonstrated to reliably track changes in information integration during working memory processing (Hosseinian et al., 2021). The results of the global efficiency analysis in the eyes-open recordings indicate a significant increase in global efficiency in the first 30 minutes for all interventions compared to the sham condition. Importantly, the increase in global efficiency was significantly greater following iTBS and tACS alone than that of the X-tACS Peak ($p<0.001$; $p=0.001$) (Figure 4a). This might partially explain why performance speed was significantly faster after iTBS and tACS protocols. The results of global efficiency during the eyes-closed recordings are shown in Figure 4b.

Insert Figure 4 here

4- Discussion

This study investigated the impact of five NIBS protocols, developed via a combination of tACS and iTBS, on inducing and stabilizing 40Hz gamma oscillations in healthy individuals. Given the potential role of targeting these oscillations as adjunct treatments for Alzheimer's disease, we also examined their association with working memory performance—a cognitive function that correlates with gamma power and is typically impaired in AD patients. In addition to examining local effects of the stimulation in the frontal lobe, this study assessed the global impact of NIBS on 40Hz gamma power across the entire brain and its functional connectivity. In what follows, we discuss major physiological and behavioral findings.

4.1. Oscillatory effects

Our major finding is that the combined protocol (40Hz iTBS phase-locked to the tACS-generated peak wave) had the strongest gamma-inducing effects in the EO recording followed by 40Hz tACS and 40 Hz iTBS. In the EC condition, all protocols involving iTBS significantly

decreased 40Hz oscillatory power compared to the sham condition but tACS slightly increased gamma and significantly boosted 40Hz oscillatory power at the 30-60-minute time window. The gamma-enhancing effects of the X-tACS peak protocol partly replicated findings of previous studies utilizing the same intervention for enhancing alpha and theta power in the EO condition. These studies demonstrated that TMS pulses applied to the peak of the tACS waves led to a stronger increase in the mid-range frequency bands in the EO condition [26,27] although a reversed result pattern (i.e., X-tACS trough protocol) was observed for the low-frequency delta band [28].

One explanation for the observed effects is that the application of iTBS at the peak of the sine wave may have induced in-phase rhythmic neuronal activity at 40Hz, thereby facilitating the entrainment effects of tACS. Previous research has demonstrated that repetitive rTMS can produce short-lasting oscillatory changes that are contingent on the frequency of the applied pulses, leading to a "phase reset" of the ongoing intrinsic oscillatory cycles [7,33] followed by short-lasting synchronization at the driving frequency [8]. In this context, the external induction of 40Hz oscillatory activity through iTBS can enhance the alignment between endogenous and externally applied oscillations, resulting in stronger entrainment effects of tACS. Additionally, some studies underscore the importance of ongoing oscillatory activity during stimulation with tACS, which can be modulated by applying iTBS in the target frequency. A study by Kemmerer and colleagues [34] showed that tACS applied to the individual alpha peak at the time led to significant modulation of oscillatory power in the target frequency, whereas tACS applied with a frequency of +/- 2Hz to the individual alpha peak or sham stimulation was less effective, emphasizing the frequency specificity of tACS for efficient online entrainment. Another study provides further supporting evidence by showing that the application of frontoparietal tACS aligned with the individual theta peak compared to the application of non-individualized 4Hz theta tACS or sham stimulation led to stronger oscillatory power in the target frequency, and also stronger effects on network synchrony in the frontoparietal network [35]. In the same line, the strongest modulation of brain rhythms was

observed at the target frequency of 40Hz, underscoring the capacity of tACS to selectively entrain endogenous oscillations in a frequency-dependent manner. Notably, oscillatory effects were also present in other frequency bands, such as theta and alpha (Figure S4), though these were less pronounced.

Another explanation concerns increased efficacy of iTBS to induce action potentials at the cortical level when the TMS pulses are applied to the peak of the tACS sine wave. Through the application of the exogenous current produced by tACS, ongoing natural oscillatory activity is aligned with the exogenous current [10]. Non-human primate studies along with computational models show that as oscillatory activity becomes aligned, the underlying neurons exhibit stronger depolarization during the positive phase (peak) of the applied current, while a hyperpolarized state is observed during the negative phase (trough) of the current [36–38]. The application of iTBS at the peak of the sine wave, corresponding to the depolarized phase of the neuronal membrane potential, might enhance action potential generation. During this phase, the membrane potential is closer to the firing threshold, which is expected to facilitate the initiation of action potentials in response to the applied stimulation. In contrast, iTBS delivered at the trough of tACS sine wave, which corresponds to the hyperpolarized phase of the membrane potential, is likely to be less effective in eliciting action potentials. In this line, Wischnewski and colleagues [39] demonstrated that the degree of cortical excitability is dependent on the phase of an ongoing oscillation. Single pulse TMS was applied over the primary motor cortex in different phases of the beta rhythm. The results showed that the application of single TMS pulses in the peak or falling phase of the oscillation led to increased MEP amplitudes. This finding supports the explanation of higher efficacy of applied TMS pulses to a depolarized phase of oscillations and the stronger effects in the target frequency for the X-tACS Peak protocol compared to the other interventional protocols.

These findings, along with previous studies, suggest that iTBS-induced 40Hz power, synchronized with 40Hz tACS, produces larger effects than other protocols by enhancing 40Hz

power at both local and network levels. Although the most pronounced increase in oscillatory power was observed in the iTBS phase-locked to the peak of the tACS wave, the specific mechanisms driving the differential effects of these interventional protocols require further investigation. A difference of the results of this study compared to our previous works [26,27] is that the stimulation effects did not transfer to the EC recording condition, while the same effect was seen for both EO and EC conditions in those studies. One possible reason for the greater intervention effects in the EO condition in the present study is the stronger high-frequency brain activity, including gamma rhythms, present when the eyes are open, compared to the general shift to lower frequencies, such as alpha rhythms, when the eyes are closed [40]. The stronger effects observed in the EO condition may be due to the higher frequency oscillatory activity during the baseline state at the time of stimulation, which occurred during rest with the eyes open. Prior research indicates that baseline oscillatory activity in the target frequency is critical for the entrainment effects of tACS [39]. Similarly, in rTMS, the baseline brain state during stimulation influences oscillatory changes [41], which may explain the enhanced effects in the EO condition.

4.2. Behavioral effects

While performance accuracy was not affected, performance speed, however, improved. This effect was not strongest for the most efficient stimulation protocol (X-tACS peak stimulation). Instead, 40Hz tACS and iTBS protocols were associated with faster behavioral performance. The null effect of interventions on performance accuracy may be attributed to the sample characteristics, as the study involved young, healthy subjects with intact executive functions, leading to a ceiling effect and generally strong task performance. On the other hand, this finding can be explained by the results obtained from the analysis of global efficiency. Previous studies have shown that global efficiency is a reliable metric to estimate the brain's ability to integrate information during a working memory paradigm [27,42]. Our results indicate that the tACS and iTBS protocols produced the

largest alterations of global efficiency within the first 30 minutes post-intervention, as observed in the eyes-open recordings. These network modifications may have facilitated improved task performance by enhancing the efficient integration of task-relevant information, which was less pronounced in the other protocols. Therefore, it can be concluded that oscillatory activity at 40Hz is not solely predictive of behavioral working memory changes. Instead, a more comprehensive analysis incorporating multiple metrics may provide a clearer understanding of the intervention effects on behavioral outcomes.

4.3. Limitations

The present study has several limitations. Firstly, it served as a proof-of-concept, examining the effects of the new stimulation protocols on oscillatory aftereffects and safety in a healthy population. Although no adverse events were reported, further research is needed to explore the stimulation effects across different healthy age groups and in clinical populations, such as those with mild cognitive impairment or Alzheimer's disease, to enhance therapeutic outcomes, particularly regarding executive functioning. Additionally, we cannot discount the possibility that visual phenomena from the tACS intervention influenced the observed 40Hz oscillatory changes in the frontal region of interest. Future studies should address these visual effects, especially with higher stimulation intensities, which may exacerbate them. Moreover, effective blinding techniques should be implemented to reduce the confounding effects of phosphenes during tACS application.

4.4. Conclusion

All protocols increased 40Hz gamma oscillations during eyes-open recordings. The most effective protocol was the X-tACS Peak protocol where the iTBS pulses were phase-locked to the peak of the tACS-generated sine wave. This finding is in accordance with findings from previous research with similar stimulation approaches. The oscillatory effects observed did largely not transfer to the behavioral level. However, tACS and iTBS led to decreased reaction times during an N-Back

working memory paradigm. These effects might be attributed to stimulation effects on the network level as it was shown by alterations of global efficiency. Future studies should focus on the underlying mechanisms of the aftereffects produced by the stimulation protocols and implications for the transfer to behavioral modulations.

References

[1] Nandi A, Counts N, Chen S, Seligman B, Tortorice D, Vigo D, et al. Global and regional projections of the economic burden of Alzheimer's disease and related dementias from 2019 to 2050: A value of statistical life approach. eClinicalMedicine 2022;51:101580. https://doi.org/10.1016/j.eclinm.2022.101580.

[2] Nichols E, Steinmetz JD, Vollset SE, Fukutaki K, Chalek J, Abd-Allah F, et al. Estimation of the global prevalence of dementia in 2019 and forecasted prevalence in 2050: an analysis for the Global Burden of Disease Study 2019. Lancet Public Health 2022;7:e105–25. https://doi.org/10.1016/S2468-2667(21)00249-8.

[3] Pardo-Moreno T, González-Acedo A, Rivas-Domínguez A, García-Morales V, García-Cozar FJ, Ramos-Rodríguez JJ, et al. Therapeutic Approach to Alzheimer's Disease: Current Treatments and New Perspectives. Pharmaceutics 2022;14:1117. https://doi.org/10.3390/pharmaceutics14061117.

[4] Woloshin S, Kesselheim AS. What to Know About the Alzheimer Drug Aducanumab (Aduhelm). JAMA Intern Med 2022;182:892. https://doi.org/10.1001/jamainternmed.2022.1039.

[5] Kobayashi M, Pascual-Leone A. Transcranial magnetic stimulation in neurology. Lancet Neurol 2003;2:145–56. https://doi.org/10.1016/S1474-4422(03)00321-1.

[6] Siebner H, Rothwell J. Transcranial magnetic stimulation: new insights into representational cortical plasticity. Exp Brain Res 2003;148:1–16. https://doi.org/10.1007/s00221-002-1234-2.

[7] Fuggetta G, Pavone EF, Fiaschi A, Manganotti P. Acute modulation of cortical oscillatory activities during short trains of high-frequency repetitive transcranial magnetic stimulation of the human motor cortex: A combined EEG and TMS study. Hum Brain Mapp 2008;29:1–13. https://doi.org/10.1002/hbm.20371.

[8] Thut G, Veniero D, Romei V, Miniussi C, Schyns P, Gross J. Rhythmic TMS Causes Local Entrainment of Natural Oscillatory Signatures. Curr Biol 2011;21:1176–85. https://doi.org/10.1016/j.cub.2011.05.049.

[9] Thut G, Miniussi C. New insights into rhythmic brain activity from TMS–EEG studies. Trends Cogn Sci 2009;13:182–9. https://doi.org/10.1016/j.tics.2009.01.004.

[10] Wischnewski M, Alekseichuk I, Opitz A. Neurocognitive, physiological, and biophysical effects of transcranial alternating current stimulation. Trends Cogn Sci 2023;27:189–205. https://doi.org/10.1016/j.tics.2022.11.013.

[11] Kasten FH, Dowsett J, Herrmann CS. Sustained Aftereffect of α-tACS Lasts Up to 70 min after Stimulation. Front Hum Neurosci 2016;10. https://doi.org/10.3389/fnhum.2016.00245.

[12] Di Lorenzo F, Ponzo V, Bonnì S, Motta C, Negrão Serra PC, Bozzali M, et al. Long-term potentiation–like cortical plasticity is disrupted in Alzheimer's disease patients independently from age of onset. Ann Neurol 2016;80:202–10. https://doi.org/10.1002/ana.24695.

[13] Jafari Z, Kolb BE, Mohajerani MH. Neural oscillations and brain stimulation in Alzheimer's disease. Prog Neurobiol 2020;194:101878. https://doi.org/10.1016/j.pneurobio.2020.101878.

[14] Casula EP, Pellicciari MC, Bonnì S, Borghi I, Maiella M, Assogna M, et al. Decreased Frontal Gamma Activity in Alzheimer Disease Patients. Ann Neurol 2022;92:464–75. https://doi.org/10.1002/ana.26444.

[15] Etter G, Van Der Veldt S, Manseau F, Zarrinkoub I, Trillaud-Doppia E, Williams S. Optogenetic gamma stimulation rescues memory impairments in an Alzheimer's disease mouse model. Nat Commun 2019;10:5322. https://doi.org/10.1038/s41467-019-13260-9.

[16] Lee HS, Ghetti A, Pinto-Duarte A, Wang X, Dziewczapolski G, Galimi F, et al. Astrocytes contribute to gamma oscillations and recognition memory. Proc Natl Acad Sci 2014;111. https://doi.org/10.1073/pnas.1410893111.

[17] Düzel E, Penny WD, Burgess N. Brain oscillations and memory. Curr Opin Neurobiol 2010;20:143–9. https://doi.org/10.1016/j.conb.2010.01.004.

[18] Howard MW. Gamma Oscillations Correlate with Working Memory Load in Humans. Cereb Cortex 2003;13:1369–74. https://doi.org/10.1093/cercor/bhg084.
[19] Lundqvist M, Rose J, Herman P, Brincat SL, Buschman TJ, Miller EK. Gamma and Beta Bursts Underlie Working Memory. Neuron 2016;90:152–64. https://doi.org/10.1016/j.neuron.2016.02.028.
[20] Dhaynaut M, Sprugnoli G, Cappon D, Macone J, Sanchez JS, Normandin MD, et al. Impact of 40 Hz Transcranial Alternating Current Stimulation on Cerebral Tau Burden in Patients with Alzheimer's Disease: A Case Series1. J Alzheimers Dis 2022;85:1667–76. https://doi.org/10.3233/JAD-215072.
[21] Murdock MH, Yang C-Y, Sun N, Pao P-C, Blanco-Duque C, Kahn MC, et al. Multisensory gamma stimulation promotes glymphatic clearance of amyloid. Nature 2024;627:149–56. https://doi.org/10.1038/s41586-024-07132-6.
[22] Zhou D, Li A, Li X, Zhuang W, Liang Y, Zheng C-Y, et al. Effects of 40 Hz transcranial alternating current stimulation (tACS) on cognitive functions of patients with Alzheimer's disease: a randomised, double-blind, sham-controlled clinical trial. J Neurol Neurosurg Psychiatry 2022;93:568–70. https://doi.org/10.1136/jnnp-2021-326885.
[23] Benussi A, Cantoni V, Cotelli MS, Cotelli M, Brattini C, Datta A, et al. Exposure to gamma tACS in Alzheimer's disease: A randomized, double-blind, sham-controlled, crossover, pilot study. Brain Stimulat 2021;14:531–40. https://doi.org/10.1016/j.brs.2021.03.007.
[24] Manippa V, Palmisano A, Nitsche MA, Filardi M, Vilella D, Logroscino G, et al. Cognitive and Neuropathophysiological Outcomes of Gamma-tACS in Dementia: A Systematic Review. Neuropsychol Rev 2023. https://doi.org/10.1007/s11065-023-09589-0.
[25] Briley PM, Boutry C, Webster L, Veniero D, Harvey-Seutcheu C, Jung J, et al. Intermittent theta burst stimulation with synchronised transcranial alternating current stimulation leads to enhanced frontal theta oscillations and a positive shift in emotional bias. Imaging Neurosci 2024;2:1–14. https://doi.org/10.1162/imag_a_00073.
[26] Hosseinian T, Yavari F, Biagi MC, Kuo M-F, Ruffini G, Nitsche MA, et al. External induction and stabilization of brain oscillations in the human. Brain Stimulat 2021;14:579–87. https://doi.org/10.1016/j.brs.2021.03.011.
[27] Hosseinian T, Yavari F, Kuo M-F, Nitsche MA, Jamil A. Phase synchronized 6 Hz transcranial electric and magnetic stimulation boosts frontal theta activity and enhances working memory. NeuroImage 2021;245:118772. https://doi.org/10.1016/j.neuroimage.2021.118772.
[28] Takahashi K, Glinski B, Salehinejad MA, Jamil A, Chang AY-C, Kuo M-F, et al. Induction and stabilization of delta frequency brain oscillations by phase-synchronized rTMS and tACS. Brain Stimulat 2024;17:1086–97. https://doi.org/10.1016/j.brs.2024.09.003.
[29] Salehinejad MA, Wischnewski M, Ghanavati E, Mosayebi-Samani M, Kuo M-F, Nitsche MA. Cognitive functions and underlying parameters of human brain physiology are associated with chronotype. Nat Commun 2021;12:4672. https://doi.org/10.1038/s41467-021-24885-0.
[30] Gratton G, Coles MGH, Donchin E. A new method for off-line removal of ocular artifact. Electroencephalogr Clin Neurophysiol 1983;55:468–84. https://doi.org/10.1016/0013-4694(83)90135-9.
[31] Oostenveld R, Fries P, Maris E, Schoffelen J-M. FieldTrip: Open Source Software for Advanced Analysis of MEG, EEG, and Invasive Electrophysiological Data. Comput Intell Neurosci 2011;2011:1–9. https://doi.org/10.1155/2011/156869.
[32] Tadel F, Baillet S, Mosher JC, Pantazis D, Leahy RM. Brainstorm: A User-Friendly Application for MEG/EEG Analysis. Comput Intell Neurosci 2011;2011:1–13. https://doi.org/10.1155/2011/879716.
[33] Lakatos P, Gross J, Thut G. A New Unifying Account of the Roles of Neuronal Entrainment. Curr Biol CB 2019;29:R890–905. https://doi.org/10.1016/j.cub.2019.07.075.
[34] Kemmerer SK, Sack AT, de Graaf TA, ten Oever S, De Weerd P, Schuhmann T. Frequency-

specific transcranial neuromodulation of alpha power alters visuospatial attention performance. Brain Res 2022;1782:147834. https://doi.org/10.1016/j.brainres.2022.147834.
[35] Zhang D-W, Moraidis A, Klingberg T. Individually tuned theta HD-tACS improves spatial performance. Brain Stimulat 2022;15:1439–47. https://doi.org/10.1016/j.brs.2022.10.009.
[36] Johnson L, Alekseichuk I, Krieg J, Doyle A, Yu Y, Vitek J, et al. Dose-dependent effects of transcranial alternating current stimulation on spike timing in awake nonhuman primates. Sci Adv 2020;6:eaaz2747. https://doi.org/10.1126/sciadv.aaz2747.
[37] Reato D, Rahman A, Bikson M, Parra LC. Effects of weak transcranial alternating current stimulation on brain activity—a review of known mechanisms from animal studies. Front Hum Neurosci 2013;7. https://doi.org/10.3389/fnhum.2013.00687.
[38] Tran H, Shirinpour S, Opitz A. Effects of transcranial alternating current stimulation on spiking activity in computational models of single neocortical neurons. NeuroImage 2022;250:118953. https://doi.org/10.1016/j.neuroimage.2022.118953.
[39] Wischnewski M, Haigh ZJ, Shirinpour S, Alekseichuk I, Opitz A. The phase of sensorimotor mu and beta oscillations has the opposite effect on corticospinal excitability. Brain Stimulat 2022;15:1093–100. https://doi.org/10.1016/j.brs.2022.08.005.
[40] Barry RJ, Clarke AR, Johnstone SJ, Magee CA, Rushby JA. EEG differences between eyes-closed and eyes-open resting conditions. Clin Neurophysiol 2007;118:2765–73. https://doi.org/10.1016/j.clinph.2007.07.028.
[41] Silvanto J, Pascual-Leone A. State-Dependency of Transcranial Magnetic Stimulation. Brain Topogr 2008;21:1–10. https://doi.org/10.1007/s10548-008-0067-0.
[42] Stanley ML, Simpson SL, Dagenbach D, Lyday RG, Burdette JH, Laurienti PJ. Changes in Brain Network Efficiency and Working Memory Performance in Aging. PLoS ONE 2015;10:e0123950. https://doi.org/10.1371/journal.pone.0123950.

CrediT authorship contribution statement

Benedikt Glinski: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Visualization, Writing – review & editing. **Mohammed Ali Salehinejad:** Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Kuri Takahashi:** Data curation, Investigation, Writing – review & editing. **Asif Jamil**: Conceptualization, Writing – review & editing. **Fatemeh Yavari:** Methodology, Writing – review & editing. **Min-Fang Kuo:** Conceptualization, Methodology, Supervision, Validation, Resources, Funding acquisition, Writing – review & editing. **Michael A. Nitsche:** Conceptualization, Supervision, Methodology, Resources, Funding acquisition, Writing– review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: MAN is a member of the Scientific Advisory Boards of Neuroelectrics and Precisis. None of the remaining authors have potential conflicts of interest to be disclosed.

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Figure legends

Figure 1: **a)** Graphical representation of the study protocol. **b)** representation of the modified iTBS protocol. The protocol was applied four times in one experimental session. The iTBS cycles started at 0, 5, 10, and 15 minutes leading to a total application of 2400 pulses in one session per stimulation side. Active stimulation in the pattern seen in the representation was applied for 190 seconds per cycle (600 pulses). **c)** Representation of 40Hz tACS with an amplitude of 1 mA peak to peak. The current was delivered for 20 minutes per session. **d)** Representation of the X-tACS Trough protocol. iTBS pulses were phase-locked to the trough (270 degrees) of the sine wave generated by tACS (represented by the arrows). The iTBS intervention was applied as described in b. **e)** Representation of the X-tACS Peak protocol. iTBS pulses were phase-locked to the peak (90 degrees) of the sine wave generated by tACS (represented by the arrows). The iTBS intervention was applied as described in b.

Figure 2: **a**) Relative time-dependent changes in 40Hz power for all stimulation protocols in the frontal ROI, compared to the baseline during **eyes-open** recordings, are shown. **b**) Relative time-dependent changes of 40Hz power in the frontal ROI are shown for all stimulation protocols in relation to the baseline for the **eyes closed** recordings. In a and b, filled symbols indicate a significant difference compared to the baseline. Asterisks above the graph indicate a significant difference compared to sham ($p<0.05$, FDR-corrected). Error bars represent the standard error of the mean. **c**) Whole brain changes in 40Hz oscillatory power are shown at four time windows following the intervention (0-30, 30-60, 60-90, and 90-120 minutes) relative to baseline during **eyes-open** recordings. T-values from post hoc tests are shown on topological graphs. Positive t-values indicate an increase in oscillatory power, while negative values indicate a decrease. Black dots indicate a significant difference from baseline ($p<0.05$, FDR-corrected). **d**) Whole brain changes in 40Hz oscillatory power across different time windows (0-30, 30-60, 60-90, and 90-120 minutes) are compared to the baseline for **eyes-closed** recordings. The t-values from post hoc tests are plotted on topographical graphs, with positive t-values indicating an increase and negative values indicating a decrease in oscillatory power. Black dots represent significant differences from the baseline ($p<0.05$, FDR-corrected). **e**) Whole brain changes of 40Hz oscillatory power for the different time windows (0-30 minutes, 30-60 minutes, 60-90 minutes, and 90-120 minutes) are presented compared to the sham stimulation for the **eyes open** recordings. t-values obtained from the post hoc tests are plotted onto the topological graphs. Positive t-values indicate an increase in oscillatory power, whereas negative values indicate a decrease in oscillatory power. Black dots indicate a significant difference compared to the sham condition ($p<0.05$, FDR-corrected). **f**) Whole brain changes of 40Hz oscillatory power for the different time windows (0-30 minutes, 30-60 minutes, 60-90 minutes, and 90-120 minutes) are presented compared to the sham stimulation for the **eyes closed** recordings. t-values obtained from the post hoc tests are plotted onto the topological graphs. Positive t-values indicate an increase in oscillatory power, whereas negative t-values indicate a decrease in oscillatory power. Black dots indicate a significant difference compared to the sham condition ($p<0.05$, FDR-corrected).

Figure 3: 3-back working memory performance following stimulation conditions (X-tACS Peak, X-tACS Trough, tACS, iTBS, and Sham). a-d illustrate performance speed-related variables. e-f illustrates performance accuracy-related variables. Data are presented as mean values±SEM. Asterisks [*] represent statistically significant differences. RT = reaction time, ms = milliseconds

Figure 4: Functional connectivity measures in the gamma band. Whole-brain global efficiency during resting state EEG—eyes open (a) and eyes closed (b) are shown. The X-axis shows epoched time points and the Y-axis shows global efficiency values. Filled symbols indicate significant differences from baseline (BL), while asterisks [*] show significant differences from the sham condition. All comparisons of epoched time windows (i.e., BL, 0-30, 30-60, 60-90, 90-120 min) are FDR corrected within and between conditions (i.e., vs sham). Panel a shows that all active stimulation protocols (X-tACS Peak, X-tACS Trough, tACS, iTBS) significantly enhanced global efficiency compared to the sham condition across all time windows during the eyes-open state. Notably, the increase in global efficiency during the first 30 minutes, when the behavioral task was conducted, was significantly greater for the tACS and iTBS protocols than for the X-tACS Peak and X-tACS Trough protocols. Panel b indicates that all active stimulation protocols (X-tACS Peak, X-tACS Trough, tACS, iTBS) significantly reduced global efficiency compared to the sham condition during the first 30 minutes and the 30-60 minute window of eyes-closed EEG recording, except for tACS, which significantly improved global efficiency in the 30-60 minute window. At all other time windows (60-90 and 90-120 minutes post-intervention), asterisks denote a significant decrease in global efficiency for the X-tACS Peak, X-tACS Trough, and iTBS protocols. Error bars represent the standard error of the mean.

Figure 1:

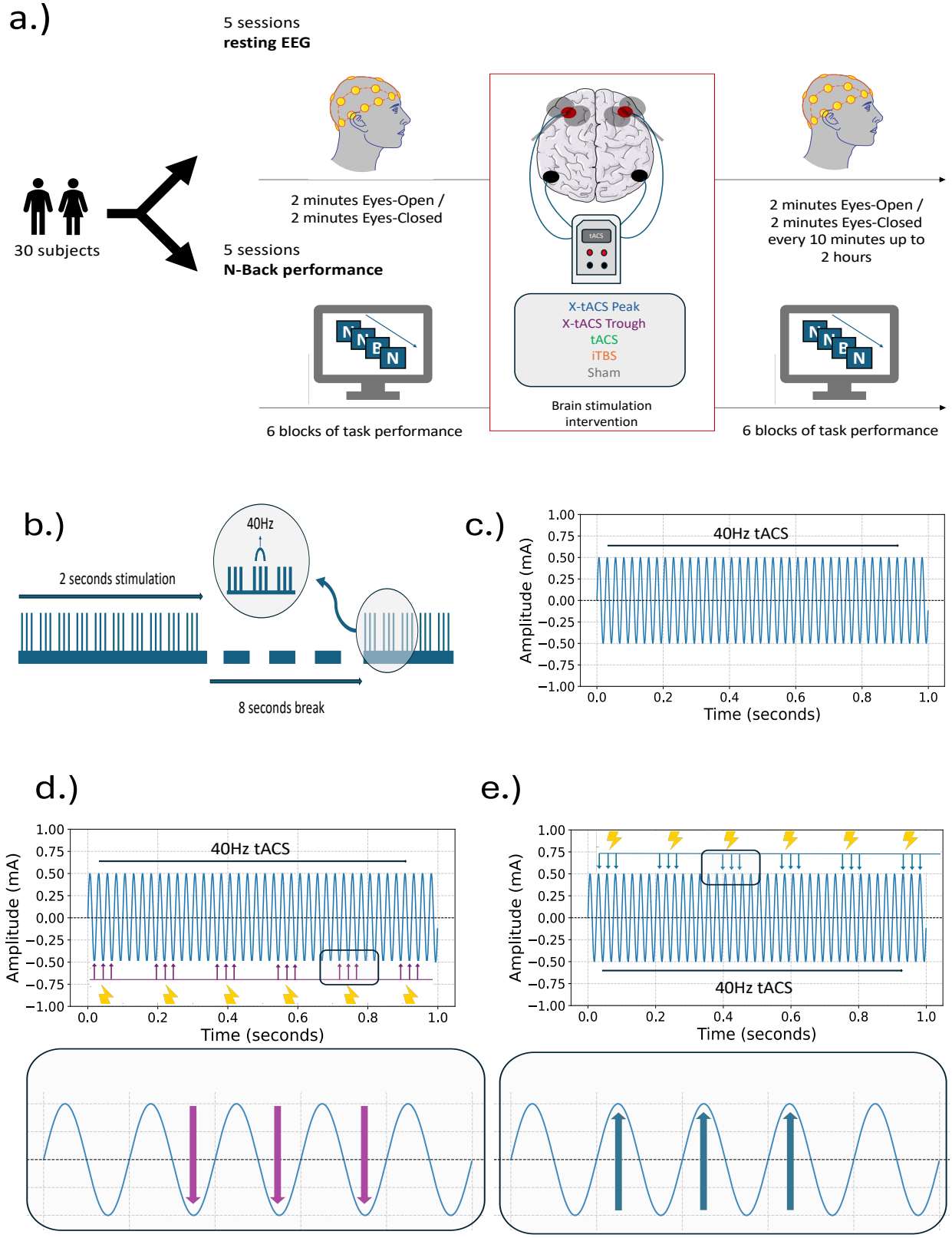

a.)
5 sessions
resting EEG
30 subjects
2 minutes Eyes-Open /
2 minutes Eyes-Closed
5 sessions
N-Back performance
6 blocks of task performance
X-tACS Peak
X-tACS Trough
tACS
iTBS
Sham
Brain stimulation
intervention
2 minutes Eyes-Open /
2 minutes Eyes-Closed
every 10 minutes up to
2 hours
6 blocks of task performance
b.)
40Hz
2 seconds stimulation
8 seconds break
c.)
40Hz tACS
Amplitude (mA)
Time (seconds)
d.)
40Hz tACS
Amplitude (mA)
Time (seconds)
e.)
40Hz tACS
Amplitude (mA)
Time (seconds)

Figure 2

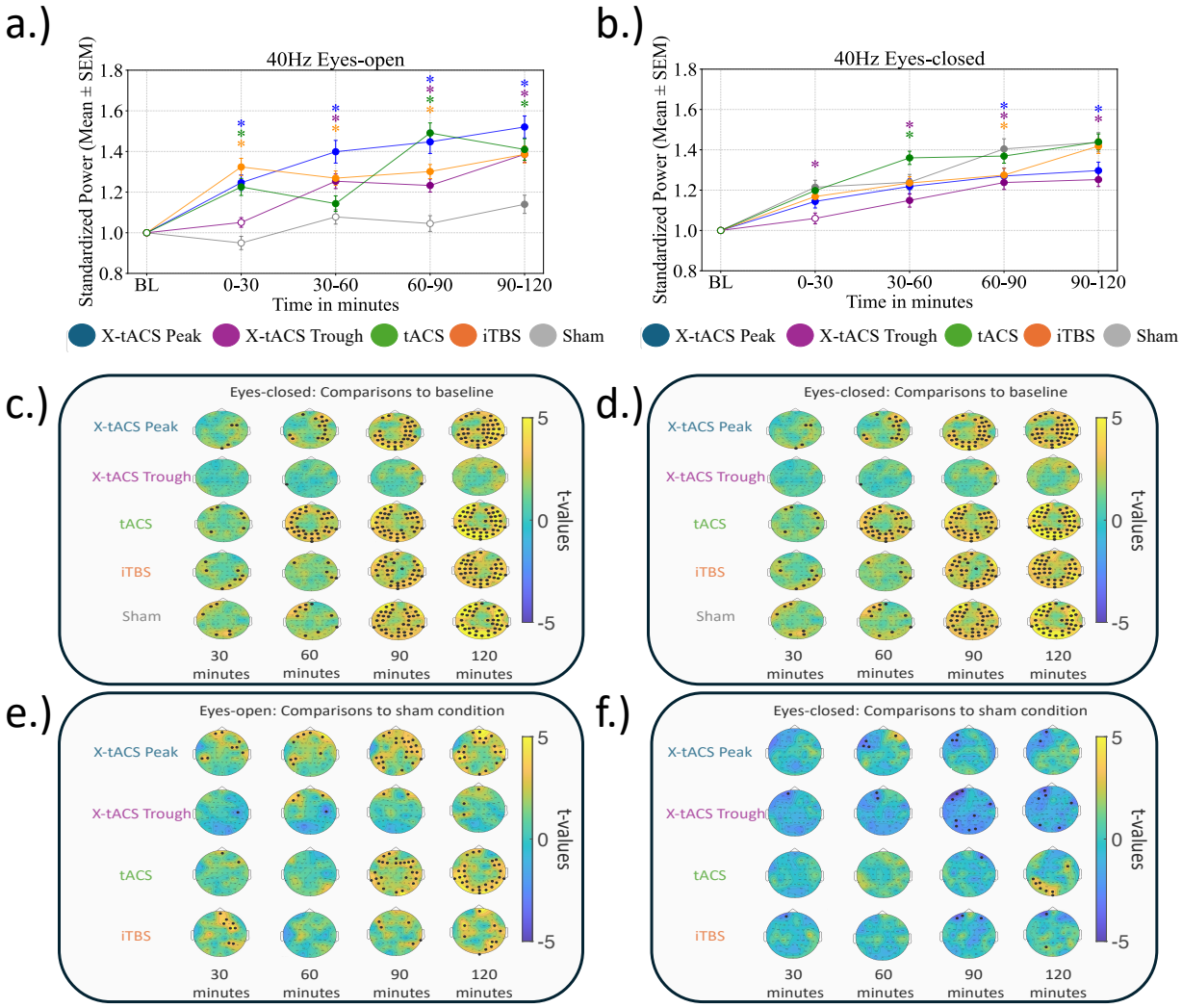

a.)
40Hz Eyes-open
Standardized Power (Mean ± SEM)
1.8
1.6
1.4
1.2
1.0
0.8
BL
0-30
30-60
60-90
90-120
Time in minutes
X-tACS Peak
X-tACS Trough
tACS
iTBS
Sham
b.)
40Hz Eyes-closed
Standardized Power (Mean ± SEM)
1.8
1.6
1.4
1.2
1.0
0.8
BL
0-30
30-60
60-90
90-120
Time in minutes
X-tACS Peak
X-tACS Trough
tACS
iTBS
Sham
c.)
Eyes-closed: Comparisons to baseline
X-tACS Peak
X-tACS Trough
tACS
iTBS
Sham
30 minutes
60 minutes
90 minutes
120 minutes
5
0
-5
t-values
d.)
Eyes-closed: Comparisons to baseline
X-tACS Peak
X-tACS Trough
tACS
iTBS
Sham
30 minutes
60 minutes
90 minutes
120 minutes
5
0
-5
t-values
e.)
Eyes-open: Comparisons to sham condition
X-tACS Peak
X-tACS Trough
tACS
iTBS
30 minutes
60 minutes
90 minutes
120 minutes
5
0
-5
t-values
f.)
Eyes-closed: Comparisons to sham condition
X-tACS Peak
X-tACS Trough
tACS
iTBS
30 minutes
60 minutes
90 minutes
120 minutes
5
0
-5
t-values

Figure 3

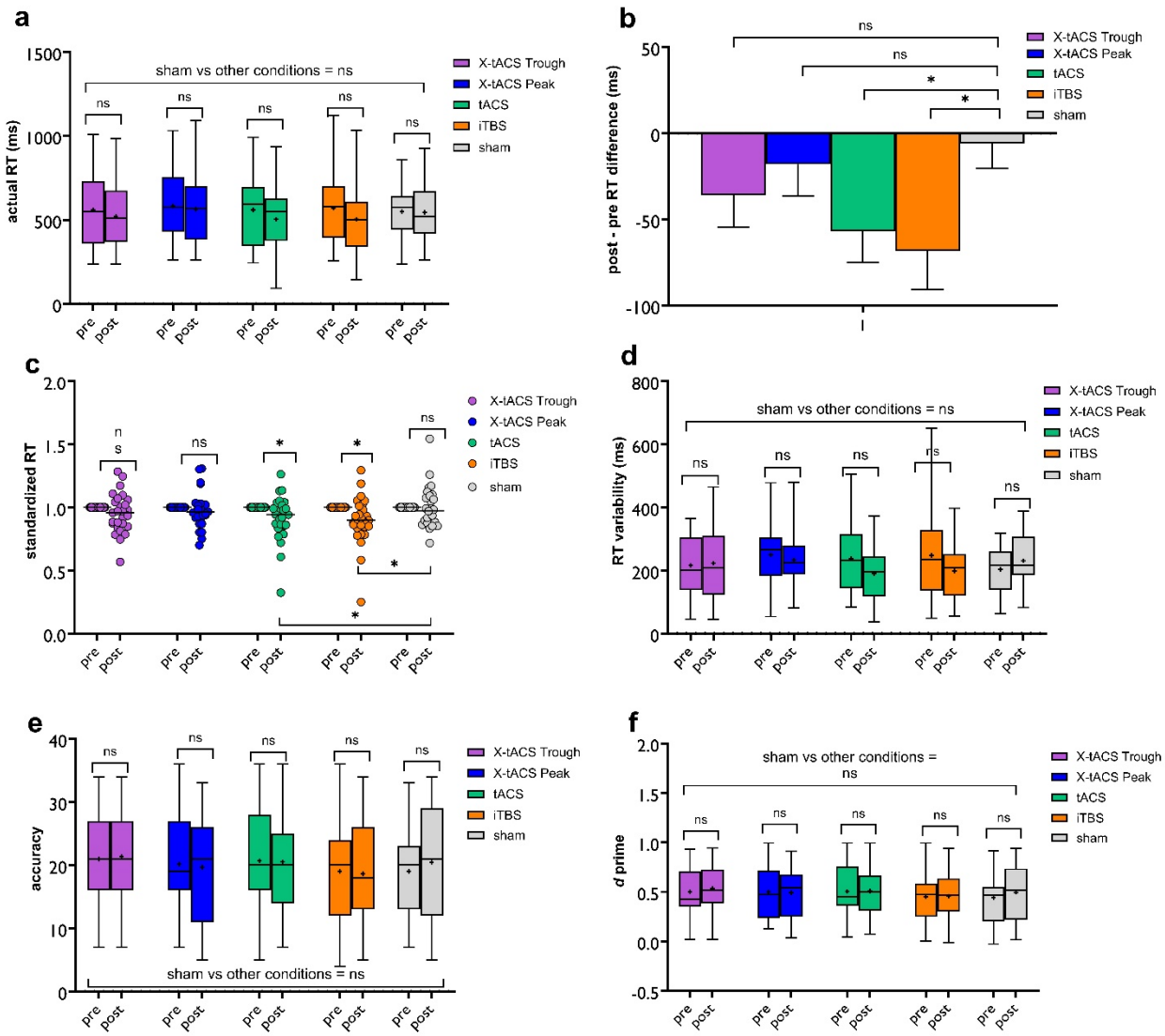
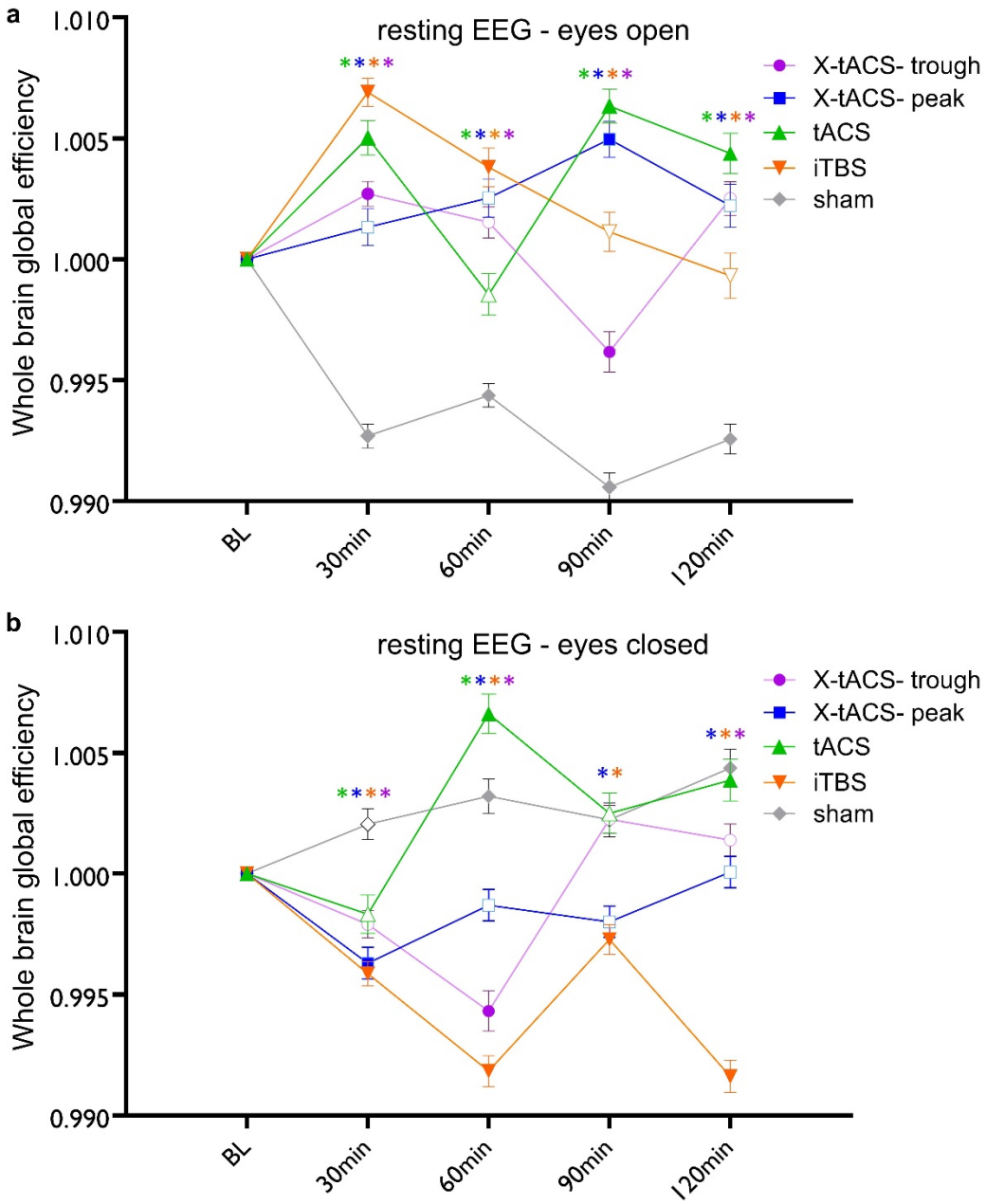

a
actual RT (ms)
sham vs other conditions = ns
ns
X-tACS Trough
X-tACS Peak
tACS
iTBS
sham
pre
post
b
post - pre RT difference (ms)
ns
*
c
standardized RT
d
RT variability (ms)
e
accuracy
f
d prime

Figure 4:


a
resting EEG - eyes open
Whole brain global efficiency
1.010
1.005
1.000
0.995
0.990
BL
30min
60min
90min
120min
X-tACS- trough
X-tACS- peak
tACS
iTBS
sham
b
resting EEG - eyes closed
Whole brain global efficiency
1.010
1.005
1.000
0.995
0.990
BL
30min
60min
90min
120min
X-tACS- trough
X-tACS- peak
tACS
iTBS
sham

Tables

Table 1:LMM results: Significant main and interaction effects of stimulation, time and electrode on 40Hz oscillatory power in the frontal ROI

Condition	Factor	d.f.	*F* value	*p* value	*ηp2*
	stimulation	4, 6979	43.90	**<0.001**	0.02
	time	4, 6978	85.41	**<0.001**	0.06
	electrode	10, 6978	5.36	**<0.001**	0.00
Eyes-open	stimulation × time	16, 6978	5.86	**<0.001**	0.01
	stimulation × electrode	40, 6978	1.02	0.43	0.00
	electrode × time	40, 6978	0.65	0.96	0.00
	stimulation × time × electrode	160, 6978	0.38	1.00	0.00
	stimulation	4, 7111	17.25	**<0.001**	0.00
	time	4, 7111	121.82	**<0.001**	0.06
	electrode	10, 7111	4.01	**<0.001**	0.00
Eyes-closed	stimulation × time	16, 7111	2.04	**<0.01**	0.00
	stimulation × electrode	40, 7111	2.81	**<0.001**	0.02
	electrode × time	40, 7111	0.83	0.77	0.00
	stimulation × time × electrode	160, 7111	0.41	1.00	0.00

Table 2: LMM results: Significant main and interaction effects of stimulation, time and electrode on 40Hz oscillatory power on the whole brain level

Condition	Factor	d.f.	*F* value	*p* value	*ηp2*
	stimulation	4, 38638	236.19	**<0.001**	0.03
	time	4, 38637	470.71	**<0.001**	0.05
	electrode	60, 38637	8.94	**<0.001**	0.01
Eyes-open	stimulation × time	16, 38637	29.10	**<0.001**	0.01
	stimulation × electrode	240, 38637	2.19	**<0.001**	0.02

	electrode × time	240, 38637	1.06	0.24	0.00
	stimulation × time × electrode	960, 38637	0.50	1.00	0.01
	stimulation	4, 38309	149.64	**<0.001**	0.02
	time	4, 38308	735.70	**<0.001**	0.07
	electrode	60, 38308	7.01	**<0.001**	0.01
Eyes-closed	stimulation × time	16, 38308	17.01	**<0.01**	0.00
	stimulation × electrode	240, 38308	2.22	**<0.001**	0.01
	electrode × time	240, 38308	1.10	0.19	0.00
	stimulation × time × electrode	960, 38308	0.55	1.00	0.01