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Registered Report

Benchmarking the effects of transcranial temporal interference stimulation (tTIS) in humans

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A B S T R A C T
Deep brain stimulation (DBS) provides clinical benefits for several neurological and psychiatric conditions. By overcoming the limitations and risks of conventional DBS, transcranial temporal interference stimulation (tTIS) has the potential to offer non-invasive stimulation of deep brain regions. However, research that investigates the efficacy of tTIS is limited to animal studies or computer simulations and its capability to modulate neural oscillations in humans has not been demonstrated so far. The method of tTIS is hypothesized to elicit its effects via neural entrainment, corresponding to the supposed mechanism of action underlying transcranial alternating current stimulation (tACS), another, more established non-invasive brain stimulation technique. Physiological effects of tACS are well established for cortical brain oscillations, but not for deep brain structures. In particular, aftereffects on the power of parieto-occipital alpha oscillations have been shown repeatedly. In a first attempt to test the efficacy of tTIS in the human brain, the current study thus seeks to compare the effects of tTIS to the well-studied aftereffect of tACS in the cortex. To investigate this research question, the current study compared MEG-recorded brain activity during a simple visual change detection task in 34 healthy subjects pre- and post-tTIS. Additionally, the effects of tTIS were contrasted to conventional tACS and a control stimulation. We expected that the parieto-occipital $\alpha$-power will increase after tTIS and tACS, in contrast to the control stimulation. Overall, no difference between the experimental groups (tTIS, tACS and control stimulation) were found regarding the source-projected increase in $\alpha$-power. Based on the results of the study two hypothesis can be made: tTIS, tACS and the control stimulation condition don’t have an effect on human
1. Introduction

Deep brain stimulation (DBS) provides clinical benefits for several neurological and psychiatric conditions like Parkinson disease (Benabid, Chabardes, Mitrofanis, & Pollak, 2009; Okun, 2012; Rodriguez-Oroz et al., 2005), treatment-resistant depression (Bewernick et al., 2010; Bewernick, Kayser, Sturm, & Schlaepfer, 2012; Lozano et al., 2008; Malone et al., 2009; Mayberg et al., 2005; Schlaepfer et al., 2008), and Alzheimer's disease (Laxton et al., 2010; Sankar et al., 2015; Smith et al., 2012). Despite its efficacy, the inevitable surgery for the implantation of the stimulation electrodes in the target brain region is accompanied with substantial risk factors (Ben-Haim, Asaad, Gale, & Eskandar, 2009; Fenoy & Simpson, 2014) and therefore only used in severe clinical conditions.

In order to overcome these limitations, transcranial electrical stimulation (tES) using temporally interfering fields has been suggested as a brain stimulation technique which is able to electrically stimulate neurons at depth non-invasively (see Grossman et al., 2017). It has to be noted, though, that tES techniques are based on a different mechanism of action (sub-threshold modulation), as compared to DBS (supra-threshold activation). Transcranial temporal interference stimulation (tTIS) can be applied by attaching two pairs of stimulation electrodes to the scalp. A high frequency alternating current is applied through each electrode pair stimulating the brain with deviating frequencies that are not in the range of regular neural frequencies but can penetrate the brain without significant impedance. The superposition of the two input currents has an envelope amplitude that change periodically at the difference frequency (Oostendorp, Delbeke, & Stegeman, 2000). Clearly the resulting stimulation frequency will be the difference of the two applied frequencies ($f_1$ and $f_2$), thus we define the target stimulation frequency as $f_{\text{diff}} = f_2 - f_1$, with $f_2 > f_1$. The target stimulation frequency is in the range relevant for a specific brain function (such as EEG rhythms). The superposition of the signal's frequencies causes an amplitude modulation oscillating at $f_{\text{diff}}$. The envelope of this modulation is maximal when the maxima of the two signals overlap in time and space (meaning $k_1 = k_2$) (Fig. 1A). That means that there will be a defined brain region in which the interference has the strongest effect (Fig. 1A). Similar to another frequently used electrical stimulation method, transcranial alternating current stimulation (tACS), tTIS is thought to elicit its effects via neural entrainment (Esmailpour, Kronberg, Reato, Parra & Bikson, 2021; Grossman et al., 2017; Herrmann & Strüber, 2017). It was shown that tTIS modulates neural firing in mice at the chosen stimulation frequency $f_{\text{diff}}$ (Grossman et al., 2017). Specifically, it was demonstrated that neurons are activated only in deep brain structures where both stimulation frequencies overlap with same strength, without stimulating the overlying cortex. When applied over the motor cortex, stimulation led to motor activity in mice like whisker- and forepaw movements (Grossman et al., 2017).

However, studies showed that the brain of mice and humans diverge in its functionality and anatomy (Miller, Horvath, & Geschwind, 2010). Consequently, results from animal studies with mice cannot be directly translated to humans.

The efficacy of tTIS in humans is not extensively studied so far. However, several simulation studies have investigated the electrical field of tTIS in the human brain. In a recent study the electric field of tTIS was compared to that of other stimulation methods, including conventional multi-electrode stimulation approaches (Huang & Parra, 2019). The authors argue that conventional tES methods and tTIS lead to similar intensities in a predefined region of interest (ROI). Another study showed that in comparison to conventional tACS, tTIS stimulates less superficial areas, and, additionally, tTIS can be used to stimulate more focal fields (Rampersad et al., 2019). However, defining parameter settings to target a predefined region of interest precisely across subjects might be challenging for tTIS and requires further research (Rampersad et al., 2019).

While the physiological and behavioral effects of tTIS in humans appear to be unclear, tACS on the other hand is a relatively well-known and established method to modulate brain oscillations and human behavior (Kanai, Chaieb, Antal, Walsh, & Paulus, 2008; Kasten, Dowsett, & Herrmann, 2016; Kasten & Herrmann, 2017; Vossen, Gross, & Thut, 2015; Zaehle, Rach, & Herrmann, 2010). TACS employs an alternating, sinusoidal current, (mostly) restricted to one frequency aiming to entrain endogenous brain oscillations (Herrmann, Rach, Neuling, & Strüber, 2013) such that they follow the external stimulation in regard to the phase and the frequency (Herrmann & Strüber, 2017). The most commonly observed effect of tACS is, however, a sustained elevation power of endogenous brain oscillation in the range of the target frequency after stimulation (Kasten et al., 2016; Kasten & Herrmann, 2017; Neuling, Rach, & Herrmann, 2013; Vossen et al., 2015; Wischnewski et al., 2019; Zaehle et al., 2010). The effects of amplitude modulated stimulation waveforms, which are thought to give rise to stimulation effect at the target region during tTIS, have recently been compared to conventional tACS in a computer simulation of a cortical network (Neghabani, Kasten, Herrmann, & Fröhlich, 2018). However, substantially higher amplitudes were needed to reach stimulation effects comparable to tACS. Consequently, it remains to be tested whether tTIS can induce meaningful modulation of neural activity in the human brain.
Given the similarity of tACS and tTIS in terms of goals and the supposed underlying mechanisms, tACS seems suitable to be employed as a benchmark to address this question. With comparable mechanisms of action, comparable online- and after-effects should be expected for both methods. Sustained changes in neural activity after tTIS are particularly interesting because these changes are comparably easy to study (no corruption of EEG/MEG signals with stimulation artifacts, see Kasten & Herrmann, 2019; Kasten, Negahbani, Fröhlich, & Herrmann, 2018). The assessment of aftereffects on the cortical level would thus allow for an easy initial assessment of the methods efficacy before targeting deeper brain structures, where stimulation effects are more difficult to measure, and little is known about the effects to be expected. Further, the prospect of aftereffects following tTIS application might be especially relevant in clinical settings, where sustained effects after few applications of tTIS are more desirable than the need for continuous stimulation to suppress symptoms.

The aim of the current study was to investigate the capability of tTIS to modulate neural oscillations in humans. Given that tTIS is assumed to work via similar mechanisms as tACS, we tested if tTIS with an envelope frequency in the alpha range is capable of modulating occipito-parietal alpha...
oscillations. Specifically, we tested if tTIS at the individual alpha frequency \((\text{faux} = 1\text{AF})\) leads to similar after effects on posterior alpha power as conventional tACS targeting the same frequency. To rule out that potential effects of tTIS can be attributed to the high frequency signals, an additional bilateral high frequency TACS control stimulation was applied. Specifically, we hypothesized that the power in the alpha band in the MEG during a simple visual change detection task have a higher increase from the pre- to the post-stimulation period after tTIS as compared to control stimulation (Hypothesis 1). The same difference was expected between the tACS- and the control-condition (Hypothesis 2). Subsequently, we expected that there is no detectable difference between tTIS and tACS (Hypothesis 3).

With regard to the last hypothesis, there may also be differences between tTIS and tACS which may complicate a comparison. For example, the effect of tACS on brain oscillations may be mediated, in part, by extraneous sensory stimulation: the direct stimulation of the skin underneath the electrodes may lead to a change in cortical activity (Asamoah, Khatoun, & Mc Laughlin, 2019). In contrast, tTIS is applied at high frequency fields that typically do not induce extraneous sensory stimulation at the same intensity as tACS (Turi et al., 2013). Thus, this could be a confounding factor in a direct comparison. We would like to emphasize though, that the primary goal of this study is to investigate whether tTIS is capable of modulating human brain oscillations. Therefore, the main comparison is tTIS versus control stimulation. As the mechanisms of tTIS seems to be similar to the mechanisms of tACS, we included a TACS condition to serve as a reference (e.g., in case tTIS would not significantly increase alpha power compared to control stimulation, a significant effect of tACS compared to control, would serve to demonstrate that the overall setup, setting and sample was in principle suited to show an effect).

While our approach does not allow to test if tTIS can modulate neuronal oscillations in deep brain structures, which is the ultimate goal of the method (Grossman et al., 2017), it comes with some advantages in terms of feasibility and interpretability of results. Due to their small size, deep brain structures are, at this point, very difficult to target on an individual level and require individually optimized stimulation montages derived from electric field modelling tools which are still under development (Huang, Datta, & Parra, 2020). At the same time, oscillatory activity in these structures is hard to assess in a non-invasive manner, bearing the risk of null results due to imprecise stimulation (Rampersad et al., 2019) or noisy measurement. In contrast, targeting cortical alpha oscillations allows to compare tTIS effects with more established approaches, a clear definition of the to be expected effects and better interpretation of null results. Failure to modulate alpha oscillations using tTIS, while being successful with tACS would indicate substantially differing underlying mechanisms of the two methods, or tTIS at conventional TES intensities being incapable of modulating neural activity in humans. At the same time, this rules out that the absence of effects is due to general problems in the study like an unfortunate brain state during stimulation, or a false assumption about to-be expected effects, which would be difficult for a deep brain target, as non-invasive stimulation approaches are not often used to target these regions. Successful modulation of alpha oscillations by tTIS would provide and initial proof of principle that the method affects neural oscillations in humans before proceeding to more challenging applications of the method in the depths of the brain.

2. Methods

2.1. Participants

G*Power (Faul, Erdfelder, Lang, & Buchner, 2007) was used to estimate the sample size by focusing on the minimum detectable effect size. The hypotheses were tested with three paired t-tests \((H1: \text{power in the alpha band was expected to have a higher power increase in the \text{TITIS condition, compared to control}}; H2: \text{power in the alpha band was expected to have a higher power increase in the \text{TACS condition, compared to control}}; H3: \text{We expected that there is no detectable difference between \text{TITIS and TACS, for detailed description see section Data Analysis}}\). In the case that the data are normal distributed, we computed one tailed, paired t-tests, if the data deviates from normal distribution we computed Wilcoxon-Rank Sign tests. As there are no effect sizes available for tTIS effects in humans, we chose an effect size, based on the smallest effect in previous TACS studies. Given the general variability of tES effects, a tTIS effect weaker than the effect size of conventional TACS would very likely not be practically meaningful. We used the smallest effect we found for the modulation of human alpha power by 20 min of tACS \((\eta^2 = .2; \text{Neuling et al., 2013})\). We used information from the manuscript by Neuling et al. (2013) \((\text{mean, SD and } n)\) to calculate Cohen’s d for the t-test. On average, participants’ increase in individual alpha power from pre to post stimulation was +48% (±/– 37%) for the tACS- and 14% (+/– 24%) for the sham-group (values were reconstructed from Fig. 4CII). To compute the effect size for the paired t-tests in G*Power, we set the correlation between the groups to 0 (as the design in Neuling et al. (2013) was between subject) and end up with \(d = .77\). We conservatively rounded this effect down to \(d = .7\), resulting in a required sample size of \(n = 32\). Repeating the same analysis for a Wilcoxon–Rank test, yielded a sample size of \(n = 34\) to achieve a power \((1-\beta)\) of .9 at an alpha level of .00667 (set to include Bonferroni correction for three comparisons).

Based on this analysis, thirty-four healthy volunteers aged between 18 and 30 years were recruited at the Carl von Ossietzky University of Oldenburg and counterbalanced for sex. In case of necessary exclusions, more participants were invited until the required 34 complete datasets were reached. Participants were eligible for the study if right-handed, without history of neurological and psychiatric disorders, non-smoking, medication free at the days of recordings and if they have normal or corrected-to-normal vision. Participants gave written informed consent and were invited at the same time of day for three separate days. Participants conducted the same task in all sessions, but with different randomized stimulation conditions (tTIS, conventional tACS and control stimulation, see section 2.4 Electrical Stimulation). Participants were debriefed immediately after the last session. The study was approved by the committee for Research Impact
assistance and Ethics of the University of Oldenburg and was conducted in accordance with the Declaration of Helsinki.

Participants were excluded from data analysis if they indicated extreme levels of tiredness, alcohol consumption on the day of the measurement or the day before, or when they did not comply task instructions.

2.2 MEG

A 306-channel whole head MEG system (Elekta Neuromag Triux, Elekta Oy, Helsinki, Finland) was used to measure neuromagnetic activity at a rate of 1 kHz. The MEG system consists of 102 magnetometers and 204 gradiometers, measuring from 102 distinct sensor locations. The MEG system is placed in a magnetically shielded room (MSR). An online bandpass filter between 0.1Hz and 330Hz was used. The participants were seated in upright position underneath the MEG helmet (68° Dewar position). Three anatomic landmarks (nasion and left and right posterior tip of tragi) and more than 200 head shape samples were digitized for co-registration of the MEG recordings with structural MRIs using a Polhemus Fastrak (Polhemus, Colchester, VT, USA). Five head position indicator (HPI) coils were used to enable continuous head-position tracking.

The individual alpha frequency (IAF) was determined based on a 3 min, resting-state, eyes open MEG recording prior to the experiment. MEG data were filtered between 1 Hz and 40 Hz, and segmented into 1 sec epochs. Fast Fourier transforms (FFTs) were computed for each of the segments (hanning window). The power peak in the averaged spectra in the alpha range (between 8 and 12 Hz) were determined from a subset of posterior sensors. The identified frequency was used as the stimulation frequency in the following experiment for tACS or tTIS. The IAF was determined separately for each session.

2.3 Anatomical MRI

Structural MRI images were needed to analyze the MEG data on source level. MRI images were acquired using a Siemens Magnetom Prisma 3T whole-body MRI scanner (Siemens, Erlangen, Germany). A T1-weighted 3-D sequence with fat suppression (MPRAGE, TR = 2000 ms, TE = 2.07 ms) with a slice thickness of .75 mm were measured.

2.4 Electrical stimulation

Simulations of the electrical field were used to verify that the tACS and tTIS electrode montages achieve similar electric fields inside the brain. To this end, the software SimNIBS 3.0 (Thielscher, Antunes, & Saturnino, 2015) was used to simulate the electrical fields of the tTIS and tACS montages. For tTIS, additionally, the maximal electric field strength was calculated over all directions according to the equations used in previous studies (Grossman et al., 2017, p. e5-e6; Rampersad et al., 2019). The tACS and tTIS simulations were calculated with 1 mA per electrode pair, resulting in comparable field strength inside the brain for both conditions. For tACS, the stimulation electrodes were positioned centered over electrode locations Cz (7 × 5 cm) and O2 (5 × 5 cm) (Fig. 1C).

Since this is a conventional electrode montage to increase the power in the occipital alpha range (Kasten, Duecker, Maack, Meiser, & Herrmann, 2019; Kasten et al., 2016), the resulting electric field was defined as the region-of-interest (ROI) for the experiment. For tTIS, one electrode pair was placed centered over electrode positions C3 (5 × 5 cm) and O1 (5 × 5 cm), and the other electrode pair was positioned centered over C4 (5 × 5 cm) and O2 (5 × 5 cm) (Fig. 1C). For tTIS, the electric field was simulated for the temporal interfering electric fields of the deviating frequencies over all directions for the envelope waveform. The resulting electrical fields correlate with $r = .98$. The mean electric field inside the ROI is for both tACS and tTIS .1 V/m with maxima at .2 V/m (Fig. 1D).

Electrical stimulation was administered via surface conductive rubber electrodes (5 × 5 cm) attached to the scalp over standardized locations following the international 10-10 system with an electrically conductive, adhesive paste (ten20 paste, Weaver & Co, Aurora, CO, USA). Positions for tACS and tTIS were chosen, based on the basis of the simulations, explained above. Locations for control stimulation condition were the same as in the tTIS condition. The tTIS frequencies $f_1$ and $f_2$ were chosen such that the carrier frequency of the modulated signal is 1000Hz. Therefore, the frequencies used for tTIS were defined with $f_1 = 1000$ Hz $– \frac{\pi}{f}$ and $f_2 = 1000$ Hz $+ \frac{\pi}{f}$, respectively. For the control tTIS stimulation condition, both electrode pairs delivered 1 mA current (peak-to-peak) at a frequency of 1000Hz. TACS was applied at the IAF.

The sinusoidal stimulation signals were digitally sampled in Matlab 2016a at a rate of 10 kHz. The digital signals were transferred to a digital analog converter (NI-USB 6251, National Instruments, Austin, TX, USA). The analog output was connected to the galvanically isolated remote inputs of two battery-operated constant current stimulators (Advanced DC Stimulator Plus, Neuroconn, Ilmenau, Germany) that do not share a common ground. The stimulators were located in an electrically shielded cabinet outside the MSR. The MRI extension-kits for the constant current stimulators (Neuroconn, Ilmenau, Germany) were used to forward the signals into the MSR. The impedances of the stimulation electrodes were kept below 20 kΩ (including two 5kΩ resistors in the stimulator cables). Prior to the experiment, participants were familiarized with potential somatosensory sensations (Matsumoto & Ugawa, 2017) by brief application of tACS or tTIS at the frequency and intensity used during the main experiment. In the time course of the experiment, participants received either 20-min of tACS at IAF with 1 mA (peak-to-peak), 20-min of tTIS at IAF ($f_1 = 1000$ Hz $– \frac{\pi}{f}$ and $f_2 = 1000$ Hz $+ \frac{\pi}{f}$) with 1 mA (peak-to-peak) per electrode pair or 20-min of control stimulation with 1 mA per electrode pair. At the beginning and end of each stimulation period, the electrical stimulation was faded in or out, respectively, with two 10-sec fade-in/fade-out intervals. After the recordings, participants filled out a questionnaire rating common adverse effects (Brunoni et al., 2011) of electrical stimulation. After the completion of all three experimental sessions, participants were informed about the order of their experimental conditions and the goal of the study.
2.5. Experimental Design

Prior to the main experiment, the IAF was determined based on a 3 min resting state MEG measurement. The main experiment started with 10 min of baseline MEG, followed by 20 min of either tACS or tTIS at their IAF, or control stimulation. All participants run through all stimulation conditions in a randomized order. The control stimulation condition was applied to rule out that stimulation effects occur due to the high frequency stimulation while also capturing time-on-task effects; e.g., natural increases in alpha band power over time, which are commonly observed with this kind of experiment (Benwell et al., 2019; Kasten et al., 2016; Neuling et al., 2013). Participants were not aware which stimulation condition they underwent on the different days. Each participant was measured with at least one week between the sessions at the same time of the day. Afterwards, 10 min of post MEG was recorded (Fig. 1B). During the recordings that lasted for a total of 40 min, participants performed a visual change detection task similar to previous studies (Kasten et al., 2016, 2019; Stecher & Herrmann, 2018; Stecher, Pollok, Strüder, Sobotka, & Herrmann, 2017; Zaehele et al., 2010) in order to warrant that participants remained awake and attentive (Kasten et al., 2016, 2019; Neuling et al., 2013). Visual stimuli were displayed using Matlab2016a, and Psychtoolbox 3 (Kleiner et al., n.d.). Stimuli were rear-projected (Panasonic PT-DS12 KE, 60 Hz refresh rate) onto a screen inside the MSR at a distance of ~100 cm. A white fixation cross was presented on a gray background at the center of the screen. At random intervals, the fixation cross rotated by 45° for a duration of 500 ms. Participants were asked to respond to the cross rotation by pressing a button with their right index finger. The underlying experimental scripts are uploaded to a public repository (see section Code Availability and Data availability).

2.6. Data analysis

MEG data were analyzed using Matlab 2018a (The Mathworks Inc., Natick, MA, USA) and the Fieldtrip toolbox (Oostenveld, Fries, Maris, & Schoffelen, 2011). R (The R Core Team, R Foundation for Statistical Computing) was used for statistical analysis.

In order to suppress external interference in the MEG data, the spatiotemporal signal space separation method (SSS), with standard settings (Lin = 8, Lout = 3, correlation limit = .98) (Taulu & Simola, 2006; Taulu, Simola, & Kajola, 2004) using MaxFilter v2.2 (ElektaNeuromag, Elekta Oy, Finland) was used. Continuous HPI signals were used to compensate for head movements. MEG data were resampled to 250 Hz and filtered between 1 and 40 Hz using a fourth-order, zero-phase Butterworth filter. Independent component analysis (ICA) was used to visually uncover and remove artifacts such as heart-beat, eye-movements, muscle activity or noise due to the cables of the stimulation electrodes. Signals were cut into 2-sec epochs and segments that still included artifacts were rejected. On each of the remaining artifact-free segments, FFTs (4-sec zero-padding, Hanning window) were computed and averaged over the first 260 segments per block.

The DICS beamformer (Gross et al., 2001) was used to project the power in the individual α-band (IAF ± 2 Hz) into source-space. Therefore, a common spatial filter was computed from the averaged cross-spectrum in the individual α-band across all segments of the pre- and post-stimulation blocks using all 306 (magnetometer and gradiometer) channels. Data was projected onto an equally spaced 6 mm grid. Single-shell head-models, that were co-registered to participants’ head position inside the MEG were used. The regularization parameter was set to λ = 1e-12. The common filters were then utilized to project the data from pre- and post-stimulation block in source space. The power difference between the pre- and post-stimulation block was computed on source level.

To test whether the power increase in the individual α-band was larger after tACS and tTIS in comparison to control stimulation, a region-of-interest (ROI) was defined. Therefore, the differences in the individual α-power between pre- and post-stimulation were calculated on source level. The ROI was defined on basis of the control stimulation condition by calculating cluster-based permutation statistics across subjects. The increase in the α-power in the ROI was compared statistically with three paired, one tailed t-tests (higher increase of alpha power for tTIS, compared to control stimulation; higher increase of alpha power for tACS, compared to control stimulation; no detectable difference between tTIS and tACS). If the data deviated from normal distribution, Wilcoxon–Rank tests were used instead. In addition, the calculation of Bayes Factor was included in the analysis. If we could not identify a significant cluster as the ROI on basis of the data of the control stimulation condition, power in the individual α-band of all sources in the brain was used for statistical comparison of the differences between the three conditions.

Additionally, we tested whether there is a difference in the alpha power on source level in the pre-stimulation period between conditions.

Due to the high spatial resolution of the data, a statistical cluster test was performed. The advantage of this test is that it assesses statistical differences between the power increase of the different stimulation conditions without defining a ROI as a prior assumption. Since it is challenging to estimate the sample size a-priori for statistical cluster analysis, the data were analyzed exploratory by testing the power differences between the three conditions with nonparametric random permutation cluster t-tests using 10 000 randomizations and Monte Carlo method to estimate P-values (corrected for multiple comparisons), implemented in the Fieldtrip toolbox.

An important aspect of tACS is frequency specificity. While not the main focus of this research, we did exploratory analyses in the neighboring frequency bands. Therefore, the same analysis pipeline, as explained above for the alpha band, was run for different frequency bands, more specifically the neighboring frequency bands beta (13–30 Hz) and theta (4–8 Hz). We included the computation of Bayes Factor for both frequency bands.
3. Results

One subject was excluded from the data analysis due to strong eye artifacts (not possible to completely remove those using ICA), resulting in a sample of 33 participants. Overall, the α-power in all experimental conditions (tTIS, tACS and control stimulation) increased from pre-to post-stimulation block (tTIS: $V = 77$, $p < .01$; tACS: $V = 111$, $p < .01$; ctrl: $V = 96$, $p < .01$, where $V$ is the test statistics, based on the pairwise difference between two groups with a Wilcoxon-singed-ranks test). Moreover, the α-source is located parieto-occipital (see Fig. 2a).

Overall, the participants had comparable performances across conditions (tTIS: $M = 93.33\%$, std:11.64; tACS: $M = 95.99\%$, std: 7.15; ctrl: $M = 94.7\%$, std = 10.49). The task performance did not differ between the conditions (tTIS vs. tACS: $p = .15$; tTIS vs. ctrl: $p = .51$; tACS vs. ctrl: $p = .52$). In line, the participants had comparable reaction times across conditions (tTIS: $M = 724.97$ ms, std: 230.1; tACS: $M = 699.4$ ms, std: 201.16; ctrl: $M = 713.89$ ms, std = 188.93). The reaction time did not differ between conditions (tTIS vs. tACS: $p = .29$; tTIS vs. ctrl: $p = .63$; tACS vs. ctrl: $p = .39$).

After stimulation, participants reported comparable sensations across stimulation conditions for ten items ($p > .05$): headache, neck pain, pain on the skin, tingling, itching, getting warm, red skin, fatigue, difficulties to concentrate, and mood swings.

3.1. Pre-registered analysis

In order to define the ROI based on the control stimulation condition, dependent samples cluster permutation t tests revealed a significant power increase in the α-band from the pre-to the post-stimulation block (permutation cluster t test, $p_{\text{cluster}} < .01$). The power increase in the α-band is located in parieto-occipital, temporal, and frontal areas. Unexpectedly, the ROI spreads over the entire cortex (orange areas in Fig. 2b).

Since the averaged source projected power increase in the α-band within the ROI depart significantly from normality for all experimental groups (Shapiro–Wilk test, tTIS: $W = .84$, $p < .05$; tACS: $W = .79$, $p < .05$, ctrl: $W = .92$, $p < .05$), Wilcoxon-singed-ranks tests were performed in order to compare the increase in the source projected α-power across experimental conditions. The source-projected power increase in the α-band within the ROI of the three experimental groups (tTIS, tACS and control stimulation) revealed no significant effects: the power increase in the α-band after tTIS is not significantly higher than the power increase in the α-band after control stimulation ($V = 226$, $p = .17$). Bayes factor ($\text{bf}_{10} = .52$) indicate anecdotal evidence for $H_0$. Moreover, the power increase in the α-band after tACS is not significantly higher than the

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**Fig. 2** – Results. A. Frequency Spectra and α-source for all experimental conditions (ctrl, tACS, tTIS). Power spectra before and after control stimulation, tACS, and tTIS, average over all gradiometer sensors and participants (top). Difference in the source projected α-power before and after control stimulation, tACS and tTIS, averaged across all subjects (bottom). B. Region-of-Interest. Statistical map contrasting post- vs. pre-power in the α-band for the control stimulation condition. Statistical map is thresholded at an α-level of .01. The cluster was used as ROI to extract the individual power increase of each subject in all experimental groups for further analysis. (left). Averaged source projected α-power increase within ROI for all experimental conditions (right).
power increase in the $\alpha$-band after control stimulation ($V = 304, p = .66$). Bayes factor ($bf_{10} = .16$) indicate substantial evidence for $H_0$. Additionally, the power increase in the $\alpha$-band of tTIS did not differ significantly to the power increase in the $\alpha$-band of tACS ($V = 204, p = .18$). Bayes factor ($bf_{10} = .45$) indicate anecdotal evidence for $H_0$.

Since the averaged source projected baseline power in the $\alpha$-band within the ROI depart significantly from normality for all experimental groups (Shapiro–Wilk test: tTIS: $W = .85, p < .001$; ctrl: $W = .82, p < .001$; tACS: $W = .85 p < .001$), Wilkoxon-signed-ranks tests were performed in order to test differences in the baseline $\alpha$-power between the groups. Neither tTIS ($V = 369, p = .12$), nor tACS ($V = 345, p = .26$) differ with respect to their $\alpha$-power during the baseline period, compared to the $\alpha$-power in the control stimulation condition. In addition, the baseline $\alpha$-power did not differ significantly between tTIS and tACS ($V = 269, p = .85$).

3.2. Exploratory analysis

A difference in $\beta$-power between baseline and post-stimulation block was observed for tACS ($t = -2.33, p = .03$), control stimulation ($t = -2.07, p = .05$), and tTIS ($t = -3.12, p = .004$). However, no effect was found between stimulation conditions: Since the averaged source projected power in the $\beta$-band across the whole brain is normally distributed across conditions (Shapiro–Wilk test, tTIS: $W = .97, p = .4$; tACS: $W = .95, p = .14$; ctrl: $W = .99, p = .96$), t-tests were performed ($ctrl$ vs. tTIS: $t = 1.58, p = .12$; $bf_{10} = .57$; $ctrl$ vs. tACS: $t = .74, p = .46$; $bf_{10} = .24$; tTIS vs. tACS: $t = .76, p = .45$, $bf_{10} = .24$).

While the $\theta$-power differs between baseline and post-stimulation block for control stimulation ($t = -2.87, p = .01$), and tTIS ($t = -2.51, p = .02$), the $\theta$-power did not differ between baseline and post-stimulation for tACS ($t = -1.18, p = .25$). The averaged source projected power in the $\theta$-band across the whole brain depart from normality across conditions (Shapiro–Wilk test, tTIS: $W = .72, p < .001$; tACS: $W = .79, p < .001$; ctrl: $W = .84, p < .001$), and, similarly to the $\beta$-band, the power increase did not differ significantly across conditions (tTIS vs. tACS: $V = 321, p = .48$, $bf_{10} = .22$; ctrl vs. tACS: $V = 307, p = .65$, $bf_{10} = .23$; ctrl vs. tTIS: $V = 218, p = .27$, $bf_{10} = .3$).

In order to account for the distribution of the power increase (not constrained by the ROI), we ran an exploratory whole-brain analysis. As visible in Fig. 2A, the distribution of the power increase on source level around the IAF $\pm 2$Hz differ between tTIS ($M = 4.6e-23$ a.u.; $std = 7.94e-23$, tACS ($M = 3.95e-23$ a.u.; $std = 6.59e-23$) and control stimulation ($M = 3.62e-23$ a.u.; $std = 5.75e-23$). However, the comparison of the source-projected power increase in the $\alpha$-band across experimental conditions by means of an independent sample random permutation cluster $t$ test revealed no significantly larger power increase, contrasted for each condition (permutation cluster $t$ test, tACS vs. ctrl: $p > .19$, tACS vs. tTIS: $p > .2$, tTIS vs. ctrl: $p > .15$).

4. Discussion

Transcranial temporal interference stimulation (tTIS) is a relatively new brain stimulation technique aiming to target deep brain structures non-invasively without stimulating the overlaying cortex. Currently, literature is mainly limited to computational studies (Huang et al., 2020; Huang & Parra, 2019; Lee, Lee, Park, & Im, 2020; Rampersad et al., 2019; von Conta et al., 2021), animal- (Esmailipour, Kronberg, Reato, Parra, & Bikson, 2021; Grossman et al., 2017) and neuronal models (Cao, Doiron, Goswami, & Grover, 2020; Cao & Grover, 2018; Howell & McIntyre, 2020; Mirzakhalili, Barra, Capogrosso, & Lempka, 2020). Therefore, the aim of the current study was to investigate the capability of tTIS to modulate neural oscillations in the human brain. In order to provide an initial proof of principle that tTIS can affect neural oscillations in humans, we studied if tTIS with an envelope frequency in the $\alpha$-range is capable of modulating occipito-parietal alpha oscillations. Specifically, we investigated if tTIS at the individual alpha frequency (IAF) leads to similar after effects on posterior $\alpha$-power as conventional tACS targeting the same frequency. An additional bilateral high frequency tACS control stimulation was applied in order to rule out that potential effects of tTIS can be attributed to the high frequency signals.

The power in the $\alpha$-band increased over time for all experimental conditions (tTIS, tACS and control stimulation), which is in line with previous studies (Kasten et al., 2016, 2019; Neuling et al., 2013; Vossen et al., 2015; Zaehle et al., 2010). In addition, the $\alpha$-source is located parieto-occipital, as expected (see Fig. 2A). Opposite to the hypotheses of the current study, the source-projected power increase in the $\alpha$-band within the ROI revealed no significant difference between the active stimulation groups (tTIS and tACS), and the control stimulation condition. However, as expected, no difference between both stimulation conditions (tTIS, tACS) were found. While bayes factor indicate anecdotal evidence for the comparison of tTIS and control stimulation ($bf_{10} = .52$), bayes factor indicate substantial evidence for the comparison of tACS and control stimulation ($bf_{10} = .16$), in favor of the $H_0$. Moreover, we confirmed that there is no effect for the neighboring frequency bands, namely $\beta$ ($12Hz–30Hz$) and $\theta$ (4Hz–8Hz). The source-projected baseline power in the $\alpha$-band did not differ across conditions.

The current study assumed that stimulation with frequencies outside the range of regular brain activity (e.g., 1000Hz) is not able to modulate human brain oscillations. Based on this assumption, the most apparent explanation for the non-existing difference regarding the increase in the $\alpha$-power across experimental conditions would be that there is no effect of both tTIS, and tACS in contrast to control stimulation. This implies that the well-established effect of tACS on $\alpha$-oscillations (Kasten et al., 2016; Kasten & Herrmann, 2017; Neuling et al., 2013; Vossen et al., 2015; Wischnewski et al., 2019; Zaehle et al., 2010) could not be replicated in the current study. Several inter- and intra-individual parameter could explain this unexpected outcome: e.g., an unfortunate brain state during brain stimulation can affect the effect of brain stimulation techniques (Bergmann, 2018). Another explanation could be that the $\alpha$-sources were not optimally targeted with the chosen electrode montage (Kasten et al., 2019). Both electrode montages of tACS and tTIS target similar parieto-occipital brain areas (both electric fields correlate with $r = .98$). Due to the similarity of the electric fields in regard to the spatial distribution, no effect can be
found for both tACS and tTIS, in case that the α-source differ across subjects. Another explanation could be that there is a mismatch regarding the stimulation frequency and the individual alpha frequency. Therefore, it might be desirable to use closed-loop stimulation protocols in order to address this issue (Karabanov, Thielscher, & Siebner, 2016; Zrenner, Belardinelli, Müller-Dahlhaus, & Ziemann, 2016). However, the major methodological difference between the current study and previous studies showing an effect of tACS on human brain oscillations in the α-range is the stimulation protocol of the control stimulation condition. Most studies used sham-, or shunt-stimulation as control condition (e.g., Kasten et al., 2019, 2016; Kasten & Herrmann, 2017; Neuling et al., 2013; Vossen et al., 2015; Zaehle et al., 2010). Therefore, the preceding interpretations of the results are strictly limited to the prior assumption that stimulation with high frequencies did not influence human brain oscillations in the α-band (similar to sham-, or shunt-stimulation). However, the mechanisms underlying stimulation with kilohertz-frequencies are poorly characterized to date (Neudorfer et al., 2023). Therefore, the control stimulation condition of the current study has to be further discussed, and compared to well-known control stimulation protocols like sham- or shunt-stimulation. The results of the current study indicate that there is no difference between the active stimulation conditions (tTIS and tACS), in contrast to the control stimulation condition. However, since tACS is a well-established brain stimulation method, the results of the current study (especially the results of tACS) can be compared to previous studies. Interestingly, the ROI (see Fig. 2b, left column) resulting from statistical comparisons within the control stimulation condition differ from expectation (based on literature (Kasten et al., 2019), the ROI was expected to be parieto-occipital, instead of a region spreading across the whole brain). Especially the comparison to a previous study by Kasten et al., 2019 (the study used a similar experiment, study design, and the same measurement device) suggest that the control stimulation condition of the current study differ from sham stimulation condition: the increase in the region where the α-power in the control stimulation condition differ significantly from pre-to post-stimulation seems to be more similar to the increase in the α-band of subjects receiving stimulation with tACS (compare Fig. 2b with Figure 4a,f in Kasten et al., 2019). Moreover, comparing the absolute source-projected power increase in the α-band with the study by Kasten et al., 2019, the increase in the α-band of the control stimulation condition of the current study seems to be more similar to the increase in the α-band of the tACS group (compare Fig. 2a with Fig. 3c–d in Kasten et al., 2019). In addition, there is a significant increase in the α-power from the pre-to the post-stimulation block across all conditions (tTIS, tACS and control stimulation), supporting the hypothesis that there might be an effect inherent in all experimental conditions in the current study. Overall, these indicate that there could be a stimulation effect in the control stimulation condition of the current study, suggesting that high frequency stimulation might lead to a modulation of brain oscillations in the α-band. However, if this hypothesis would be confirmed in further studies, the main assumption of the mechanism of tTIS would be violated: the overlaying cortex would always be co-stimulated during tTIS (at least with a carrier frequency of 1000Hz), in addition to the target region where both electric fields overlap with same strength. In order to investigate this issue, further studies have to investigate the effects of different carrier frequencies (e.g., 1000Hz and 2000Hz), as well as a sham control condition.

However, there is evidence against the assumption that the control stimulation condition modulated the brain in the same way as the tACS and tTIS condition. First, the increase in alpha-power could be mediated by a natural increase in alpha power over time in all conditions. This time-on task effect is commonly observed during comparable experimental setups (Benwell et al., 2019; Kasten et al., 2016; Neuling et al., 2013). Second, some studies question the ability of weak electric fields (as used in the current study) to exert neuro-modulatory effects (Vörösákos et al., 2018). Nevertheless, several previous studies show an effect of tACS with peak-to-peak amplitudes of 1 mA (e.g., Kasten et al., 2016; Kasten et al., 2019; Neuling et al., 2013). Moreover, there is evidence that tES with smaller electric fields in the range of 3 V/m can already influence the timing of spike activity (Johnson et al., 2020; Krause, Vieira, Csorba, Filly, & Pack, 2019). Third, the frequency content of the tACS and control stimulation condition is different. Therefore, it could be questioned if both stimulation techniques can lead to similar temporal changes in brain oscillations. There is, however, both supportive evidence that there is a neuro-modulatory effect in all experimental conditions, and evidence suggesting a lack of neuro-modulatory effects in all conditions. Unfortunately, the results of the current study are inconclusive in this regard. Future studies on the effects of tTIS should incorporate both an established inactive control stimulation (e.g., sham stimulation applied for only few seconds) as well as a high frequency control at the carrier frequency of tTIS.

Recently, a few studies investigated the effects of tTIS in humans (Zhu et al., 2022; Wessel et al., 2021; Ma et al., 2021). Studies showed that tTIS can modulate neural oscillations in the motor cortex (Ma et al., 2021), and can strengthen the functional connectivity between the primary and the secondary motor cortex (Zhu et al., 2022). It has to be noted, though, that the current study can only be compared with the published studies to a limited extent due to different stimulation current densities, different measurement devices (e.g. fMRI), different target areas, and different carrier frequencies.

Some limitations of the current findings need to be considered. As mentioned above, the major methodological difference between the current study and previous studies using brain stimulation techniques is the stimulation protocol of the control condition: Most studies use as control conditions sham/shunt (e.g. Kasten et al., 2019, 2016; Kasten & Herrmann, 2017; Neuling et al., 2013; Vossen et al., 2015; Zaehle et al., 2010). Moreover, it is known that stimulation with higher frequencies need higher stimulation intensities in order to achieve comparable effects (Negahbani et al., 2018; Thiele, Zaehle, Haghikia, & Ruhnau, 2021). In addition, the current study used a fixed carrier frequency of 1000Hz. It
might be beneficial to adjust the carrier frequency individually that the carrier frequency is not a harmonic of the frequency of interest in the $\alpha$-band. A huge discussion in brain stimulation research is to account for interindividual differences. Therefore, it might be beneficial to adjust the electrode montages on individual level to target the $\alpha$-source.

Since this is one of the first studies investigating after effects of tTIS in humans, replication studies are inevitable. Moreover, further research is needed in order to investigate possible effects of high frequency stimulation on brain oscillations in the $\alpha$-band.

Data availability statement

The complete raw MEG datasets are available online (https://osf.io/ncj3q/). The approved and published Stage 1 protocol is available online (https://osf.io/9ts27/). The conditions of our ethics approval do not permit public archiving of anonymized raw MRI data, since faces could be reconstructed. Readers seeking access to the data should contact the corresponding author CSH. Access will be granted to named individuals in accordance with ethical procedures governing the reuse of sensitive data. Specifically, requestors must complete a formal data sharing agreement to obtain the data. Alternatively, the post-processed head- and sourcemodels required to reproduce the analysis are available in the repository along with the code used for segmentation. We report how we determined our sample size, all data exclusions (if any), all inclusion/exclusion criteria, whether inclusion/exclusion criteria were established prior to data analysis, all manipulations, and all measures in the study.

Code availability statement

All experimental- and analysis-scripts are available online (https://osf.io/ncj3q/).

Author contributions

JC, FHK, BCB, AA and CSH conceived the study; KS supervised the study from a technical point of view; JC programmed the experiment; JC acquired data, JC analyzed the data, all authors wrote the manuscript.

Open practices

The study in this article earned Open Materials and Preregistered badges for transparent practices. Materials and data for the study are available at https://osf.io/ncj3q/

Declaration of competing interest

CSH holds a patent on brain stimulation. KS is the manufacturer of the advanced DC stimulator plus (Neuroconn, Ilmenau, Germany). JC, FHK, BCB and AA declare no competing interests.

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Supplementary data

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REFERENCES


