Visually Evoked 40 Hz Gamma Activity Enhanced by Transcranial Electrical Stimulation

Authors

Hainke, Laura[1,2,3] (laura.hainke@tum.de) *
Spitschan, Manuel[3,4,5] (manuel.spitschan@tum.de)
Priller, Josef[1,6,7,8] (josef.priller@tum.de)
Taylor, Paul[2] (paul.taylor@psy.lmu.de)
Dowsett, James[9] (james.dowsett@stir.ac.uk)

* corresponding author

Affiliations

[1] Department of Psychiatry and Psychotherapy, TUM School of Medicine and Health, Technical University of Munich, Munich, Germany
Ismaninger Str. 22, 81675 Munich, Germany
[2] Department of Psychology, LMU Munich, Munich, Germany
Leopoldstr. 13, 80802 Munich, Germany
[3] Department of Health and Sport Sciences, TUM School of Medicine and Health, Technical University of Munich, Munich, Germany
Georg-Brauchle-Ring 60, 80992 Munich, Germany
[4] Translational Sensory & Circadian Neuroscience, Max Planck Institute for Biological Cybernetics, Tübingen, Germany
Max-Planck-Ring 8, 72076 Tübingen, Germany
[5] TUM Institute of Advanced Study (TUM-IAS), Technical University of Munich, Garching, Germany
Lichtenbergstraße 2 a, 85748 Garching bei München, Germany
[6] Neuropsychiatry, Charité – Universitätsmedizin Berlin and DZNE, Berlin, Germany
Charitépl. 1, 10117 Berlin, Germany
[7] University of Edinburgh and UK DRI, Edinburgh, UK
Old College, South Bridge, Edinburgh EH8 9YL, United Kingdom
[8] German Center for Mental Health (DZPG), Munich, Germany
[9] University of Stirling, Stirling, UK
University of Stirling, Stirling, FK9 4LA, Scotland UK

Abstract

Background

Non-invasive Visual Stimulation (VS) and Transcranial Electrical Stimulation (TES) can modulate neuronal oscillations, including gamma activity at 40 Hz, which is relevant for cognition and disrupted in dementia. Combining both techniques may increase effects, but simultaneously recording Electroencephalography (EEG) activity poses several challenges, so this approach is untested.

Objectives

We predicted that combined TES and VS in the lower gamma band would increase Steady-State Visually Evoked Potential (SSVEP) amplitude during and after stimulation, but only when targeting visual areas and at closely matching stimulation frequencies.

Methods

We administered combined VS and TES and simultaneously measured effects on EEG gamma activity in healthy participants. In experiment 1 (N=25), VS and TES frequencies were closely matched at ~40 Hz, and TES sites varied between occipito-central, centro-occipital (reversed polarity), and centro-frontal. In experiment 2 (N=25), occipito-central TES was applied at ~40 Hz, and VS frequency varied between 35, 40, and 45 Hz. Every 5-minute VS+TES trial was preceded and followed by a VS-only baseline trial. Electrical artifacts were removed using adaptive template subtraction.

Results

TES enhanced gamma SSVEP amplitudes most when applied to occipital and central sites, compared to frontal. Enhancement only occurred when TES and VS frequencies closely matched at ~40 Hz, not when VS was slower (35 Hz) or faster (45 Hz) than TES. The effect was present during, not after, TES.

Conclusion

Multimodal visual and electrical stimulation evokes stronger oscillatory gamma activity than visual alone. Non-invasive gamma stimulation against cognitive decline in dementia may benefit from this optimised approach.

Keywords

Transcranial Electrical Stimulation (TES); Steady-State Visually Evoked Potential (SSVEP); Electroencephalography (EEG); 40 Hz gamma; flicker; Non-Invasive Brain Stimulation (NIBS)

Abbreviations

EEG: Electroencephalography
NIBS: Non-Invasive Brain Stimulation
SSVEP: Steady-State Visually Evoked Potential
SSVEPamp: SSVEP peak-to-peak amplitude in microvolt
TES: Transcranial Electric Stimulation
VS: Visual Stimulation

1. Introduction

Visual Stimulation (VS) and Transcranial Electrical Stimulation (TES) are two forms of non-invasive brain stimulation (NIBS) which, if applied periodically, can modulate neuronal oscillations and associated brain functions safely and effectively. Oscillations in the gamma band (30-100 Hz, especially at 40 Hz) are of particular interest due to their role in perception and memory (Herrmann et al., 2010) and because they are disrupted in older age, cognitive impairment, and Alzheimer's Disease (Güntekin et al., 2022). By modulating gamma activity, both VS and TES at this frequency are promising approaches to causally investigate memory functions (Hanslmayr et al., 2019) and to intervene against cognitive decline in dementia (Guan et al., 2022; Nissim et al., 2023; Shu et al., 2024; Strüber & Herrmann, 2020; Traikapi & Konstantinou, 2021). If multisensory stimulation yields larger effects than one modality alone (Blanco-Duque et al., 2023), combining sensory and electrical modalities is also likely to increase effects, but this approach is untested.

To examine how VS and TES might interact, it is necessary to measure brain activity concurrently. However, recording Magneto- or Electroencephalography (M/EEG) during TES is challenging due to electrical artifacts, which are several orders of magnitude larger than the neuronal signal of interest (Kasten & Herrmann, 2019; Noury et al., 2016). Measuring gamma activity also poses particular challenges, as endogenous gamma power is relatively low, and ocular or muscular artifacts can be confounded with neuronal signals (Hipp & Siegel, 2013). Some prior work combining periodic VS, oscillatory TES, and the measurement of Steady-State Visually Evoked Potentials (SSVEPs) at lower frequencies up to 10 Hz points toward feasibility (Dowsett et al., 2020; Ruhnau et al., 2016), but it also highlights the complexities of such designs. Stimulation protocols and analysis parameters can significantly impact the study outcome (Dowsett & Herrmann, 2016). In sum, there is no prior evidence on how combined VS and TES in the gamma band can influence neuronal gamma activity, which stimulation parameters to choose, or whether concurrently measured EEG outcomes in this frequency range can be reliably interpreted in the face of physiological and electrical artifact sources.

The objective of the present study was to close these knowledge gaps. We administered combined periodic visual and electrical stimulation in the lower gamma band and concurrently measured effects on oscillatory EEG activity. This study aimed to clarify A) if obtaining clean EEG data during gamma-band pulsed transcranial direct current stimulation, a form of oscillatory TES, is feasible; B) if gamma TES can augment SSVEPs elicited by gamma VS; and C) how different VS frequencies and TES sites modify the outcome. We addressed these questions through two experiments. In the first one, VS and TES frequencies were closely matched (40 / 39.9 Hz; see Stimulation), and TES sites varied between occipito-central, centro-occipital (reversed polarity), and centro-frontal. Here, we aimed to rule out effects of electrical currents on the retina or peripheral nerves as confounds (Asamoah et al., 2019; Kar & Krekelberg, 2012) and investigate potential impacts of current direction (Balslev et al., 2007). In the second experiment, occipito-central TES was applied at 39.9 Hz, and VS frequency varied between 35, 40, and 45 Hz. Here, we tested for frequency specificity and further confirmed successful artifact removal (Dowsett et al., 2020). Assuming that EEG data acquired during multimodal stimulation could be reliably processed and interpreted, we hypothesised that combined TES and VS in the lower gamma band would increase SSVEP amplitude during and

after stimulation, but only if TES was applied between occipital and central areas and at a rate closely matching VS frequency.

2. Methods

2.1 Design

We hypothesised that to modulate SSVEP amplitude through combined TES and VS in the lower gamma band, TES should be applied between occipital and central sites to target visual brain areas, and the frequency of both stimulation modalities should be closely matched (Dowsett et al., 2020; Ruhnau et al., 2016). Each of these factors (site and frequency) was tested in a different experiment. For those subjects who participated in both experiments (see below), sessions were at least one month apart. Each experiment included three blocks in a pseudo-randomized order, with every trial of combined TES and VS preceded and followed by a baseline VS-only trial with the same frequencies (Figure 1). In experiment 1, VS was set to 40 Hz and TES to 39.9 Hz in all trials (see Stimulation), and TES sites varied per block (Figure 2). We selected anodal O2 / cathodal Cz (“occipito-central”) for the main condition, anodal Cz / cathodal O2 (“centro-occipital”) as a reversed polarity condition, and anodal Cz / cathodal Fz (“centro-frontal”) as a site control (Herring et al., 2019). In experiment 2, 39.9 Hz TES was applied to occipito-central sites and VS frequency varied per block. 40 Hz VS was the main condition, equivalent to experiment 1; 35 and 45 Hz were frequency controls.

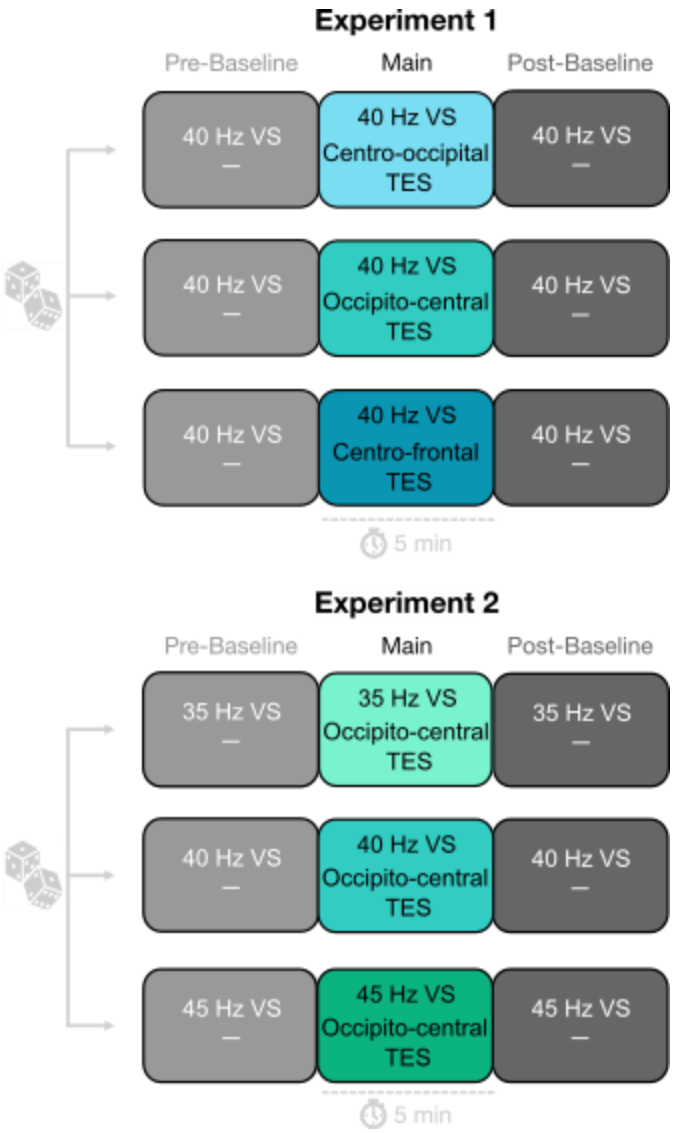
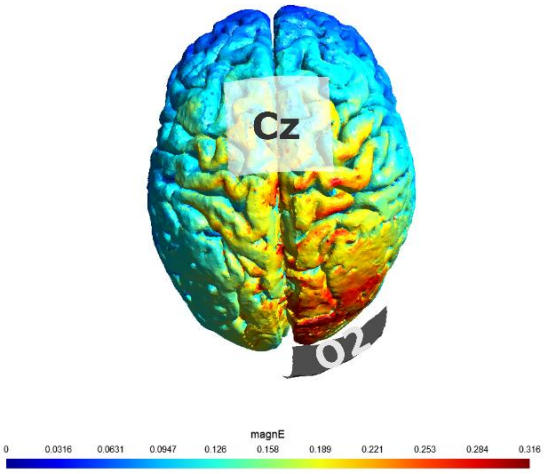
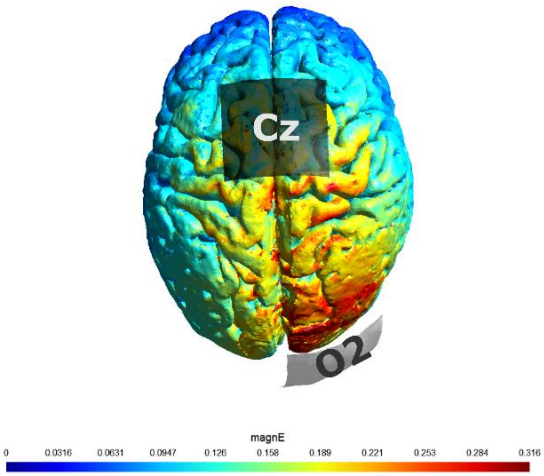


Figure 1 Experimental protocol.
Structure of blocks and trials in both experiments. The experimental condition, occipito-central TES + 40 Hz VS, was included in both experiments. VS = Visual Stimulation; TES = Transcranial Electrical Stimulation (at 39.88 Hz). Dice represent block randomisation.

Centro-occipital



Occipito-central



Centro-frontal

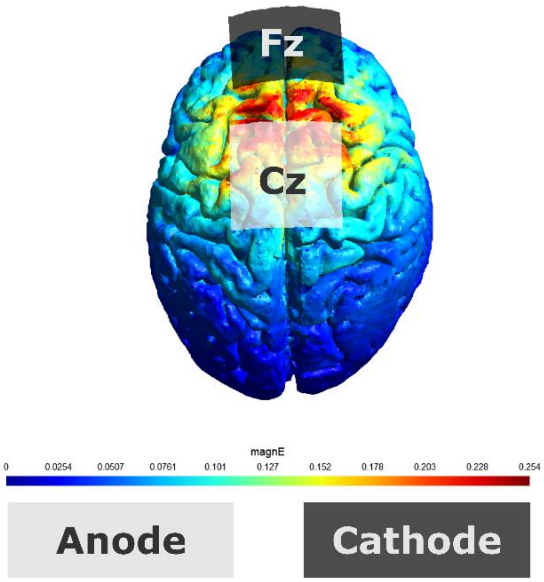


Figure 2 Simulated current flows.
Simulation of TES effects for the three montages used in experiment 1, computed with SimNIBS software (Saturnino et al., 2019). Brain models display modeled current distributions during a tDCS pulse. The centro-occipital montage is equivalent to the occipito-central one with reversed polarity; they look the same, because the models display the absolute amplitude of the electric field. magnE = absolute magnitude of the electric field in V/m.

2.2 Sample

With approval by the LMU Munich Psychology ethical committee, we recruited healthy adult participants. Written informed consent was obtained from all participants for being included in the study. The exclusion criteria were a history of seizures or epilepsy (also for first-degree relatives) and any psychiatric or neurological condition, as well as colour blindness, assessed with an Ishihara test. 25 subjects participated in experiment 1; 21 agreed to participate in experiment 2 as well, for which an additional four subjects were recruited to match the first experiment's sample size. In both experiments (*N*=25 each), the indicated gender matched the sex assigned at birth for all subjects; 21 subjects were right-handed, three were left-handed, and one was ambidextrous, respectively. The age range was 18 - 28 years, with a mean age of 23.1 years in experiment 1 (18 female / 7 male) and a mean age of 23.8 years in experiment 2 (17 female / 8 male).

2.3 Stimulation

Transcranial electrical stimulation (TES) was administered using the neuroConn DC-Stimulator Plus (neuroCare Group GmbH, Munich, Germany). Using the "remote input" function, the stimulation waveform was delivered to the stimulator with a digital-to-analogue converter. The O2, Cz, and Fz positions were selected according to EEG 10-20 standards based on a previous study (Dowsett et al., 2020); current flows simulated using one author's brain scans and SimNIBS software (Saturnino et al., 2019) are shown in Figure 2. During TES trials, TES was administered continuously for five minutes. The pulsed transcranial direct current stimulation oscillated between 0 and 0.8 mA in a square-wave pattern with a duty cycle of 50 %. The frequency of 39.9 Hz, just below the VS frequency of interest at 40 Hz, allowed for phase shifts between TES and VS, such that the VS and TES gradually drifted out of phase, and all relative phases were stimulated in one block. We stimulated across all phases as the optimal stimulation phase is unknown; it might be different across participants, and there may be multiple phases eliciting an effect. Cycling through all phases allows us to measure the average effect (Dowsett et al., 2020). Moreover, any TES artifacts remaining after data processing would be distributed across phase bins, averaging to zero given enough trials.
Visual stimulation (VS) was delivered through a custom-made system powered by a microcontroller, with light-emitting diodes embedded in a mask blocking off any external light. Stimulation ("flicker") was temporally modulated in a square-wave pattern with a duty cycle of 50 %. Participants' eyes were closed to match other experimental setups of ours during sleep. Narrowband red light with a peak wavelength of 605 nm ensured transmittance through closed eyelids (Bierman et al., 2011); the target illuminance was 175 photopic lux at eye level, a value chosen based on pilot tests. In every trial, the temporally modulated light alternated between

ON and OFF periods of 8.325 s, respectively. This duration ensured that for the primary frequency of interest of 40 Hz, the TES and VS cycles drifted apart long enough to cover all phases in one ON-period. OFF-periods were used for TES artifact removal.

2.4 Protocol

Each session began with TES and EEG setup (impedances < 10 kOhm) and gradual habituation to TES and VS. Three blocks were recorded in a pseudo-randomized order, with three five-minute trials each (VS-only pre-baseline, combined VS + TES, VS-only post-baseline). During trials, participants were instructed to keep their eyes closed and centred. They took short breaks between blocks with the mask lifted and eyes open.

2.5 EEG Acquisition & Processing

EEG data were recorded with the BrainAmp system (Brain Products GmbH, Gilching, Germany) at a 5 kHz sampling rate. The ground was placed on the left earlobe, the reference on the right earlobe, and one active electrode approximately at P2 - the midpoint between the two main TES electrodes at O2 and Cz, where the electric artifact was expected to be smallest. A DC correction was applied before every block to prevent signal saturation by the TES electric charge. Data were divided into segments time-locked to the flicker, with a length depending on VS frequency of 22.2 ms (45 Hz), 25 ms (40 Hz), or 28.8 ms (45 Hz).
Electrical artifacts caused by the TES were removed based on the following adaptive template subtraction method (Dowsett et al., 2020): For each segment in one 8.325 s VS-ON period, at least five segments in the subsequent 8.325 s VS-OFF period are selected, for which the phase of the electrical artifact matches the one in the segment to be cleaned. Concretely, each segment of VS-OFF data is tested by subtracting it from the segment in the VS-ON period to be cleaned; remaining artifacts would be visible in the first differential of the resulting segment as spikes at the time points of the steep rise or fall of the square-wave TES signal. In this case, the algorithm replaces this segment with another one from the VS-OFF period. This process is repeated until at least five suitable segments, which optimally match the shape of the electrical artifact, are found. Then, the five segments with the closest match are averaged into a template. The remaining segments are looped through and added to the template only if the overall match is improved. The final template is then subtracted from the segment to clean. Notably, it includes only the electrical artifact, not the SSVEP, since the template segments are taken from a VS-OFF period. This procedure was repeated for all segments in VS-ON periods. Then, after rejecting segments with a peak-to-peak amplitude > 200 µV, SSVEPs were computed by averaging all cleaned segments within one condition. SSVEP peak-to-peak amplitude in µV (SSVEPamp) was the primary analysis outcome.

2.6 Statistical Analysis

A priori, we expected 39.9 Hz TES to increase SSVEP amplitudes compared to pre-baseline only when 40 Hz VS was combined with occipito-central or centro-occipital TES (online stimulation effect). We also hypothesised that post-baseline amplitudes would be higher than pre-baseline in those conditions (stimulation after-effect). One-tailed permutation tests were

performed for directed hypotheses with a significance criterion of α=0.05 and Cohen's *d* as effect size. Two-tailed permutation tests were performed for expected null effects and post-hoc tests. Additionally, we computed Inverse Bayes factors to quantify the level of evidence in favour of null hypotheses. Exploratory analyses compared the occipito-central TES + 40 Hz VS condition between both experiments (test-retest reliability) through SSVEP correlation coefficients and a Pearson correlation of TES minus pre-baseline amplitude differences.

3. Results

3.1 39.9 Hz TES increases the amplitude of 40 Hz SSVEPs, with effect magnitudes depending on TES sites

In experiment 1 (*N*=25; Figure 3 A, B), VS was always set to 40 Hz, and TES sites varied. SSVEP amplitudes (SSVEPamp) during occipito-central TES were significantly higher (p < .0001) than at VS-only pre-baseline (Mean difference = 1.62 µV; 95 % CI = [1.06 ; 2.19] µV; d = 0.94). SSVEPamp during centro-occipital TES, with the same electrode positions but reversed polarity, was also significantly higher (p < .0001) than at pre-baseline (Mean difference = 1.72 µV; 95 % CI = [1.06 ; 2.38] µV; d = 0.91). Contrary to expectations, SSVEPamp was also significantly higher (p = .012) during centro-frontal TES than at pre-baseline (Mean difference = 0.78 µV; 95 % CI = [0.21 ; 1.35] µV; d = 0.5). Post-hoc comparisons with [$SSVEPamp_{TES}$ - $SSVEPamp_{pre\text{-}baseline}$] as the dependent variable confirmed that effects were larger in occipito-central than centro-frontal TES (p = .03; Mean difference = 0.84 µV; 95 % CI = [0.09 ; 1.6] µV; d = 0.44) and larger in centro-occipital than centro-frontal TES (p = .026; Mean difference = 0.94 µV; 95 % CI = [0.17 ; 1.71] µV; d = 0.45). Effects did not significantly differ between occipito-central and centro-occipital TES (p > .05, Mean difference = -0.1 µV; 95 % CI = [-0.89 ; 0.7] µV).

3.2 Occipital TES only enhances SSVEPs when TES and VS frequencies approximately match

In experiment 2 (*N*=25; Figure 3 C, D), TES was always applied at occipito-central sites at the same near-40 Hz frequency, and VS frequency varied. Replicating the outcome of experiment 1, when combining near-40 Hz TES with 40 Hz VS, SSVEPamp was significantly higher (p < .0001) than at pre-baseline (Mean difference = 1.86 µV; 95 % CI = [1.01 ; 2.71] µV; d = 0.85). As predicted, when applying TES together with 35 Hz VS, SSVEPamp was not significantly higher (p > .05) than at pre-baseline (Mean difference = -0.17 µV; 95 % CI = [-0.45 ; 0.1] µV). The same was true for 45 Hz VS (p > .05; Mean difference = 0.02 µV; 95 % CI = [-0.08 ; 0.12] µV). Post-hoc comparisons with [$SSVEPamp_{TES}$ - $SSVEPamp_{pre\text{-}baseline}$] as the dependent variable confirmed that effects were larger in 40 Hz than 35 Hz (p = .001; Mean difference = 2.03 µV; 95 % CI = [1.11 ; 2.95] µV; d = 0.96) and larger in 40 Hz than 45 Hz (p = .001; Mean difference = 1.84 µV; 95 % CI = [0.98 ; 2.7] µV; d = 0.91). Inverse Bayes Factors indicated moderate evidence for the null hypothesis for 35 Hz (BF_{10} = 5.6) and 45 Hz (BF_{10} = 7.94; Supplementary Materials).

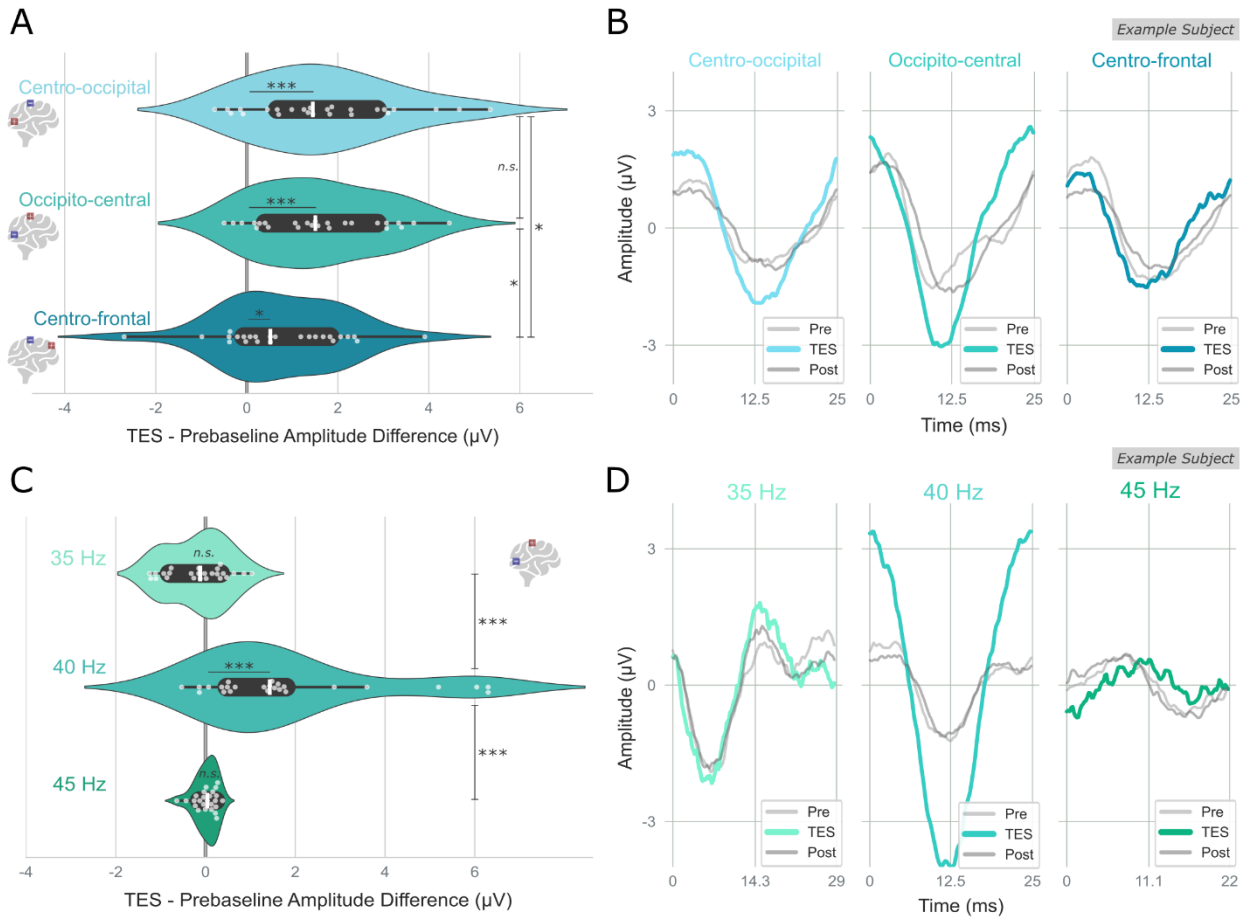


Figure 3 Results and example SSVEPs.
A) Experiment 1: Near-40 Hz TES increased 40 Hz SSVEP amplitudes over pre-baseline, most strongly at occipito-central and centro-occipital, but also slightly at centro-frontal sites. White lines = medians. B) Experiment 1: example SSVEPs from a single subject. C) Experiment 2: Near-40 Hz occipito-central TES increased 40 Hz SSVEP amplitudes over pre-baseline, replicating experiment 1; it did not increase 35 Hz or 45 Hz SSVEP amplitudes. White lines = medians. D) Experiment 2: example SSVEPs from a single subject.

3.3 Mixed results of TES after-effects on SSVEPs

In experiment 1 (*N*=25), post-baseline amplitudes after occipito-central TES were significantly lower (p = .041) than at pre-baseline (Mean difference = -0.14 μV; 95 % CI = [-0.3 ; 0.02] μV; d = -0.11). The same was true for centro-occipital TES (p = .013; Mean difference = -0.15 μV; 95 % CI = [-0.28 ; -0.03] μV; d = -0.14). After centro-frontal TES, as hypothesised, post-baseline amplitudes did not significantly differ (p > .05) from pre-baseline (Mean difference = -0.09 μV; 95 % CI = [-0.28 ; 0.1] μV). In experiment 2 (*N*=25), post-baseline amplitudes after occipito-central TES and 40 Hz VS did not significantly differ (p > .05) from pre-baseline (Mean difference = -0.02 μV; 95 % CI = [-0.13 ; 0.1] μV); the finding of experiment 1 was thus not replicated. After administering TES combined with 35 Hz VS, post-baseline amplitudes did not significantly differ (p > .05) from pre-baseline (Mean difference = -0.21 μV; 95 % CI = [-0.5 ; 0.07] μV). In the 45

Hz condition, however, post-baseline amplitudes were significantly lower (p = .044) than at pre-baseline (Mean difference = -0.11 µV; 95 % CI = [-0.22 ; 0.0] µV; d = -0.22).

3.4 40 Hz SSVEPs were stable across pre-baseline trials and variable across TES trials

Lastly, exploratory analyses compared the main condition, occipito-central TES + 40 Hz VS, across both experiments (N=21). On average, pre-baseline 40 Hz SSVEP waveforms before occipito-central TES were strongly correlated between experiments (Mean r = 0.75, SD = 0.26; Figure S5A). By contrast, 40 Hz SSVEP waveforms during occipito-central TES were only weakly correlated between experiments (Mean r = 0.11, SD = 0.59; Figure S5B). On an intra-individual level, this points to a high stability of 40 Hz SSVEPs, and a high variability of the effect of occipito-central TES on 40 Hz SSVEPs. Baseline-corrected SSVEP amplitudes under TES [$SSVEPamp_{TES}$ - $SSVEPamp_{pre\text{-}baseline}$] were moderately correlated between experiments but failed to reach statistical significance (p = .06, r = .41).

4. Discussion

The present study combined periodic electrical and visual stimulation to modulate neuronal gamma-band activity concurrently recorded with EEG. We demonstrated that 39.9 Hz Transcranial Electrical Stimulation (TES) increases visually evoked 40 Hz gamma activity. Notably, TES was more effective when applied between occipital and central areas, as opposed to frontal areas. Thus, the effect cannot be entirely attributed to electrical currents in the retina or the scalp, since more current reached the retina in the frontal control condition and comparable current levels reached the scalp in all conditions. Occipital-central stimulation was most effective with both anodal and cathodal setups, so the electrical current likely reached visual areas similarly in both conditions. We also showed that the TES frequency must approximately match the Visual Stimulation (VS) frequency to increase SSVEP amplitudes since significant effects of 39.9 Hz TES were only found on 40 Hz SSVEPs, not 35 Hz or 45 Hz. That result matches previous findings on lower alpha-band frequencies (Dowsett et al., 2020).

4.1 Implications

Pulsed TES combined with periodic VS modulated gamma activity more strongly than the visual modality alone. This finding is highly relevant given the widespread interest in gamma stimulation to causally investigate associated cognitive functions such as memory (Hanslmayr et al., 2019), the role of gamma activity in general brain health (Başar, 2013), and new therapeutic avenues for neurodegenerative conditions, including Alzheimer's Disease (Blanco-Duque et al., 2023). The present study opens the door for future research targeting higher neuronal frequencies with multimodal TES, VS, and EEG based on the optimised parameters determined here – i.e., occipital-central TES sites and matching stimulation frequencies. One limitation of our method is that we could not assess whether combined TES and VS elicits stronger gamma activity than TES alone, as our artifact removal and time series analysis approach was centred around SSVEPs. However, using SSVEPs makes it possible to reliably measure online stimulation effects. In our data recorded in relatively short trials, for example, focusing analyses

on offline after-effects would have yielded null results while missing out on the clear positive online effects. Moreover, online EEG-TES can help distinguish between responders and non-responders: in our sample, despite the significant group average, some subjects had low SSVEP amplitudes in the experimental occipito-central TES condition compared to pre-baseline in both experiments (Figure S6). Overall, this study takes electrical artifact removal in EEG a step further, a matter we need to settle if we are to explore the full potential of NIBS combined with EEG (Bergmann et al., 2016; Kasten & Herrmann, 2019; Miniussi et al., 2012; Taylor, 2018).

4.2 Methodological Advances

This study's first noteworthy methodological contribution is the artifact removal procedure that builds on previous adaptive template subtraction protocols (Dowsett et al., 2020), confirming its effectiveness in a higher frequency range. It is agnostic to VS frequency, so any major artifacts would have been visible in the 35 Hz and 45 Hz SSVEPs, but this was not the case. Moreover, all phases between 40 Hz VS and 39.9 Hz TES were covered for all VS frequencies. This means that the effect at 40 Hz VS is neither due to residual artifacts, equally likely in all VS frequencies and phases, nor to TES pulses arriving at certain 40 Hz VS phases only and linearly summing up. The 40 Hz effect we found is most likely attributable to the TES hitting the optimal VS phase(s) multiple times in a row, resulting in resonance (Supplementary Materials; Figure S4). Another strength of this study is the use of SSVEPs: with a high Signal-to-Noise Ratio and large number of short data segments, confounders such as muscular or ocular activity, which share the gamma frequency range, average out. Particularly, the square-wave VS stimuli used are optimal to elicit SSVEPs more effectively (Panitz et al., 2023). Lastly, square-wave TES facilitates TES artifact removal and neuromodulation: steeper electric field gradients have been shown to have greater effects on neuronal firing rates (Fröhlich & McCormick, 2010), and some computational models suggest square wave stimulation could enhance neural oscillations more effectively (Sherfey et al., 2018).

4.3 Future Directions

Some unexpected findings from this study could be followed up in future research. First, though smaller than the effect of TES targeted at visual areas and less consistent across participants (Figure S1), the effect of centro-frontal TES on SSVEP amplitude was still significant. Further studies could clarify whether A) centro-frontal regions are indirectly entrained by VS or TES, and this signal is mixed with the SSVEP, resulting in an amplitude increase; B) the effect arises from combined stimulation of the visual cortex and retina; or C) centro-frontal montage currents still reach the visual cortex and amplify the SSVEP. Second, TES after-effects were either null or negative, with lower 40 Hz SSVEP amplitudes in post- than pre-baseline for occipito-central and centro-occipital TES – though, in experiment 2, this effect was only significant for 45 Hz, not 40 Hz. As was speculated in a previous study (Ruhnau et al., 2016), TES may have disrupted endogenous oscillations' phase synchrony to some degree. Trials may have been too short to elicit positive after-effects but long enough to cause fatigue, as the post-baseline was always the last trial of the 15-min block and some subjects reported reduced alertness, which is known to dampen gamma SSVEPs (Gulbinaite et al., 2019). Lastly, the high variability of TES effects

on SSVEPs over time despite the stability of baseline 40 Hz SSVEPs (Figure S5) is intriguing. Conceivably, slight variations in TES electrode positions, plastic changes in cortical structure, or different subject states could explain some of this variability – which is worth investigating, considering the translational potential of such stimulation techniques (Guerra et al., 2020; Polanía et al., 2018). This potential should be explored with particular emphasis on cognitive function and age, a moderator of endogenous gamma activity and entrainment (Bakhtiari et al., 2023; Murty et al., 2020).

4.4 Conclusion

We demonstrated that applying pulsed Transcranial Electrical Stimulation to visual areas at a frequency closely matching that of periodic Visual Stimulation enhances visually evoked 40 Hz gamma activity concurrently measured with EEG. Our data showed that this form of multimodal stimulation can modulate neuronal gamma activity more strongly than the visual modality alone, while ruling out electrical or physiological confounds. This finding points to an exciting translational avenue: independently, both visual and electrical stimulation protocols to modulate gamma oscillations are known to be safe, tolerable, and clinically relevant in Mild Cognitive Impairment and Alzheimer's Disease (Blanco-Duque et al., 2023; Manippa et al., 2023). Experts are calling for the next generation of neurotechnology to be scalable, broadly applicable, and effective in modulating brain activity and plasticity (Amunts et al., 2024; Ploner et al., 2023). Our data pose multimodal gamma stimulation as a strong candidate and encourage further research to establish its potential as a therapeutic intervention.

5. Data Availability

Upon publication, all anonymised data will be shared on Open Science Framework, and study materials and code will be shared on GitHub.

6. Author Contributions

Conceptualisation: LH, JD, PT, MS
Data Curation: LH
Formal Analysis: LH, JD, PT, MS
Funding Acquisition: JD, PT, JP
Investigation: LH, JD
Methodology: LH, JD, PT
Project Administration: JD, PT
Resources: JD, PT
Software: LH, JD
Supervision: JD, PT, MS, JP
Validation: LH, JD
Visualisation: LH
Writing – Original Draft Preparation: LH
Writing – Review & Editing: JD, PT, MS, JP

7. Funding

This work was supported by the Deutsche Forschungsgesellschaft (DFG) grants [DO 2460/1-1] to JD and [TA857/3-2] to PT, the NEUROTECH Innovation Network (IGSSE) to JP, and the Max Planck Society to MS.

8. Declarations of Interest

LH, MS, PT, and JD report no conflicts of interest. JP received consulting fees from Uniqure, has a patent on Epo variants, is on the advisory board of the Sinapps2 study, and is a board member of the following societies: DGPPN, DGGPP, DZPG, DZNE.

9. Acknowledgements

The authors would like to acknowledge Joshi Walzog and Oliver Holder from the Max-Planck Institute for Biological Cybernetics electronics workshop for developing the visual stimulation system and all participants.

References

Amunts, K., Axer, M., Banerjee, S., Bitsch, L., Bjaalie, J. G., Brauner, P., Brovelli, A., Calarco, N., Carrere, M., Caspers, S., Charvet, C. J., Cichon, S., Cools, R., Costantini, I., D'Angelo, E. U., De Bonis, G., Deco, G., DeFelipe, J., Destexhe, A., … Zaborszky, L. (2024). The coming decade of digital brain research: A vision for neuroscience at the intersection of technology and computing. *Imaging Neuroscience*, *2*, 1–35. https://doi.org/10.1162/imag_a_00137

Asamoah, B., Khatoun, A., & Mc Laughlin, M. (2019). tACS motor system effects can be caused by transcutaneous stimulation of peripheral nerves. *Nature Communications*, *10*(1), 266.

Bakhtiari, A., Petersen, J., Urdanibia-Centelles, O., Ghazi, M. M., Fagerlund, B., Mortensen, E. L., Osler, M., Lauritzen, M., & Benedek, K. (2023). Power and distribution of evoked gamma oscillations in brain aging and cognitive performance. *GeroScience*. https://doi.org/10.1007/s11357-023-00749-x

Balslev, D., Braet, W., McAllister, C., & Miall, R. C. (2007). Inter-individual variability in optimal current direction for transcranial magnetic stimulation of the motor cortex. *Journal of Neuroscience Methods*, *162*(1–2), 309–313.

Başar, E. (2013). A review of gamma oscillations in healthy subjects and in cognitive impairment. *International Journal of Psychophysiology*, *90*(2), 99–117. https://doi.org/10.1016/j.ijpsycho.2013.07.005

Bergmann, T. O., Karabanov, A., Hartwigsen, G., Thielscher, A., & Siebner, H. R. (2016). Combining non-invasive transcranial brain stimulation with neuroimaging and electrophysiology: Current approaches and future perspectives. *NeuroImage*, *140*, 4–19. https://doi.org/10.1016/j.neuroimage.2016.02.012

Bierman, A., Figueiro, M. G., & Rea, M. S. (2011). Measuring and predicting eyelid spectral transmittance. *Journal of Biomedical Optics*, *16*(6), 067011. https://doi.org/10.1117/1.3593151

Blanco-Duque, C., Chan, D., Kahn, M. C., Murdock, M. H., & Tsai, L.-H. (2023). Audiovisual gamma stimulation for the treatment of neurodegeneration. *Journal of Internal Medicine*. https://doi.org/10.1111/joim.13755

Dowsett, J., & Herrmann, C. S. (2016). Transcranial Alternating Current Stimulation with Sawtooth Waves: Simultaneous Stimulation and EEG Recording. *Frontiers in Human Neuroscience*, *10*. https://www.frontiersin.org/articles/10.3389/fnhum.2016.00135

Dowsett, J., Herrmann, C. S., Dieterich, M., & Taylor, P. C. J. (2020). Shift in lateralization during illusory self-motion: EEG responses to visual flicker at 10 Hz and frequency-specific modulation by tACS. *European Journal of Neuroscience*, *51*(7), 1657–1675. https://doi.org/10.1111/ejn.14543

Fröhlich, F., & McCormick, D. A. (2010). Endogenous electric fields may guide neocortical network activity. *Neuron*, *67*(1), 129–143.

Guan, A., Wang, S., Huang, A., Qiu, C., Li, Y., Li, X., Wang, J., Wang, Q., & Deng, B. (2022). The role of gamma oscillations in central nervous system diseases: Mechanism and treatment. *Frontiers in Cellular Neuroscience*, *16*. https://www.frontiersin.org/articles/10.3389/fncel.2022.962957

Guerra, A., López-Alonso, V., Cheeran, B., & Suppa, A. (2020). Variability in non-invasive brain stimulation studies: Reasons and results. *Neuroscience Letters*, *719*, 133330. https://doi.org/10.1016/j.neulet.2017.12.058

Gulbinaite, R., Roozendaal, D. H. M., & VanRullen, R. (2019). Attention differentially modulates the amplitude of resonance frequencies in the visual cortex. *NeuroImage*, *203*, 116146. https://doi.org/10.1016/j.neuroimage.2019.116146

Güntekin, B., Erdal, F., Bölükbaş, B., Hanoğlu, L., Yener, G., & Duygun, R. (2022). Alterations of resting-state Gamma frequency characteristics in aging and Alzheimer's disease. *Cognitive Neurodynamics*. https://doi.org/10.1007/s11571-022-09873-4

Hanslmayr, S., Axmacher, N., & Inman, C. S. (2019). Modulating Human Memory via Entrainment of Brain Oscillations. *Trends in Neurosciences*, *42*(7), 485–499. https://doi.org/10.1016/j.tins.2019.04.004

Herring, J. D., Esterer, S., Marshall, T. R., Jensen, O., & Bergmann, T. O. (2019). Low-frequency alternating current stimulation rhythmically suppresses gamma-band oscillations and impairs perceptual performance. *NeuroImage*, *184*, 440–449. https://doi.org/10.1016/j.neuroimage.2018.09.047

Herrmann, C. S., Fründ, I., & Lenz, D. (2010). Human gamma-band activity: A review on cognitive and behavioral correlates and network models. *Neuroscience & Biobehavioral Reviews*, *34*(7), 981–992. https://doi.org/10.1016/j.neubiorev.2009.09.001

Hipp, J., & Siegel, M. (2013). Dissociating neuronal gamma-band activity from cranial and ocular muscle activity in EEG. *Frontiers in Human Neuroscience*, *7*. https://www.frontiersin.org/articles/10.3389/fnhum.2013.00338

Kar, K., & Krekelberg, B. (2012). Transcranial electrical stimulation over visual cortex evokes phosphenes with a retinal origin. *Journal of Neurophysiology*, *108*(8), 2173–2178.

Kasten, F. H., & Herrmann, C. S. (2019). Recovering Brain Dynamics During Concurrent tACS-M/EEG: An Overview of Analysis Approaches and Their Methodological and Interpretational Pitfalls. *Brain Topography*, *32*(6), 1013–1019. https://doi.org/10.1007/s10548-019-00727-7

Manippa, V., Palmisano, A., Nitsche, M. A., Filardi, M., Vilella, D., Logroscino, G., & Rivolta, D. (2023). Cognitive and Neuropathophysiological Outcomes of Gamma-tACS in Dementia: A Systematic Review. *Neuropsychology Review*. https://doi.org/10.1007/s11065-023-09589-0

Miniussi, C., Brignani, D., & Pellicciari, M. C. (2012). Combining Transcranial Electrical Stimulation With Electroencephalography: A Multimodal Approach. *Clinical EEG and Neuroscience*, *43*(3), 184–191. https://doi.org/10.1177/1550059412444976

Murty, D. V. P. S., Manikandan, K., Kumar, W. S., Ramesh, R. G., Purokayastha, S., Javali, M., Rao, N. P., & Ray, S. (2020). Gamma oscillations weaken with age in healthy elderly in human EEG. *NeuroImage*, *215*, undefined-undefined. https://doi.org/10.1016/j.neuroimage.2020.116826

Nissim, N. R., Pham, D. V. H., Poddar, T., Blutt, E., & Hamilton, R. H. (2023). The impact of gamma transcranial alternating current stimulation (tACS) on cognitive and memory processes in patients with mild cognitive impairment or Alzheimer's disease: A literature review. *Brain Stimulation*. https://doi.org/10.1016/j.brs.2023.04.001

Noury, N., Hipp, J. F., & Siegel, M. (2016). Physiological processes non-linearly affect electrophysiological recordings during transcranial electric stimulation. *NeuroImage*, *140*, 99–109. https://doi.org/10.1016/j.neuroimage.2016.03.065

Panitz, C., Gundlach, C., Boylan, M. R., Keil, A., & Müller, M. M. (2023). Higher amplitudes in steady-state visual evoked potentials driven by square-wave versus sine-wave contrast modulation – A dual-laboratory study. *Psychophysiology*, *n/a*(n/a), e14287. https://doi.org/10.1111/psyp.14287

Ploner, M., Buyx, A., Gempt, J., Gjorgjieva, J., Müller, R., Priller, J., Rückert, D., Wolfrum, B., & Jacob, S. N. (2023). Reengineering neurotechnology: Placing patients first. *Nature Mental Health*, *1*(1), 5–7. https://doi.org/10.1038/s44220-022-00011-x

Polanía, R., Nitsche, M. A., & Ruff, C. C. (2018). Studying and modifying brain function with non-invasive brain stimulation. *Nature Neuroscience*, *21*(2), 174–187. https://doi.org/10.1038/s41593-017-0054-4

Ruhnau, P., Keitel, C., Lithari, C., Weisz, N., & Neuling, T. (2016). Flicker-Driven Responses in Visual Cortex Change during Matched-Frequency Transcranial Alternating Current Stimulation. *Frontiers in Human Neuroscience*, *10*. https://www.frontiersin.org/articles/10.3389/fnhum.2016.00184

Saturnino, G. B., Puonti, O., Nielsen, J. D., Antonenko, D., Madsen, K. H., & Thielscher, A. (2019). SimNIBS 2.1: A Comprehensive Pipeline for Individualized Electric Field Modelling for Transcranial Brain Stimulation. In S. Makarov, M. Horner, & G. Noetscher (Eds.), *Brain and Human Body Modeling: Computational Human Modeling at EMBC 2018* (pp. 3–25). Springer International Publishing. https://doi.org/10.1007/978-3-030-21293-3_1

Sherfey, J. S., Ardid, S., Hass, J., Hasselmo, M. E., & Kopell, N. J. (2018). Flexible resonance in prefrontal networks with strong feedback inhibition. *PLoS Computational Biology*, *14*(8), e1006357.

Shu, I.-W., Lin, Y., Granholm, E. L., & Singh, F. (2024). A Focused Review of Gamma Neuromodulation as a Therapeutic Target in Alzheimer's Spectrum Disorders. *Journal of Psychiatry and Brain Science*, *9*(1). https://doi.org/10.20900/jpbs.20240001

Strüber, D., & Herrmann, C. S. (2020). Modulation of gamma oscillations as a possible therapeutic tool for neuropsychiatric diseases: A review and perspective. *International Journal of Psychophysiology*, *152*, 15–25. https://doi.org/10.1016/j.ijpsycho.2020.03.003

Taylor, P. C. J. (2018). Combining NIBS with EEG: What Can It Tell Us About Normal Cognition? *Current Behavioral Neuroscience Reports*, *5*(2), 165–169. https://doi.org/10.1007/s40473-018-0153-x

Traikapi, A., & Konstantinou, N. (2021). Gamma Oscillations in Alzheimer's Disease and Their Potential Therapeutic Role. *Frontiers in Systems Neuroscience*, *15*. https://www.frontiersin.org/articles/10.3389/fnsys.2021.782399