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ORIGINAL RESEARCH

Perceptibility and Pain Thresholds in Low- and High-Frequency Alternating Current Stimulation: Implications for tACS and tTIS

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Abstract

Transcranial electrical stimulation (tES) has emerged as a promising tool for neuromodulation, but its application is often limited by the discomfort associated with higher stimulation intensities. Newer variants like transcranial temporal interference stimulation (tTIS) utilize high-frequency alternating currents (≥ 500 Hz) to penetrate deeper brain regions while mitigating perceptual discomfort. This study sought to examine sensation and pain thresholds across various stimulation frequencies of alternating currents, aiming to explore the boundaries of comfortable intensities. Additionally, we sought to evaluate the efficacy of an anesthetizing topical cream in increasing participant comfort and potentially extending the range of tolerable stimulation levels. We recruited 37 participants and applied alternating current stimulation to the head at various frequencies (10 Hz, 20 Hz, 500 Hz, 1000 Hz, and 2000 Hz) to determine intensity-dependent perception and pain thresholds. Additionally, thresholds were determined under the influence of a topical anesthetic. Our findings confirm that as stimulation frequency increases, perceptibility decreases, with higher frequencies allowing a manyfold increase in stimulation intensity before becoming perceptible or causing pain. Additionally, the anesthetizing cream was efficacious in further reducing perceptibility and pain sensations across all frequencies. This study lays the groundwork for future research by establishing comfortable limits for stimulation intensities, particularly in the context of high-frequency stimulation. The reduced perceptibility of high-frequency stimulation, coupled with the effectiveness of anesthetizing creams, enables the administration of higher stimulation intensities for more potent neuromodulatory interventions without causing discomfort.

Keywords Transcranial alternating current stimulation (tACS) · Transcranial electrical stimulation (tES) · Transcranial temporal interference stimulation (tTIS) · Somatosensory perception · Nociception · Topical skin anesthetization

Introduction

Transcranial electrical stimulation (tES) holds significant promise for treating various psychological and neurological conditions. Research has explored its potential applications in depression (Arul-Anandam & Loo, 2009; Vanderhasselt et al., 2015), stroke (Convento et al., 2016; Khan et al., 2022; Solomons & Shanmugasundaram, 2019), and fatigue (Linnhoff et al., 2019; Shirvani et al., 2021) among others (Cho et al., 2022). Beyond therapeutic applications, there is growing interest in leveraging this technology for neurofacilitation to, for example, enhance motoric performance (Chang, 2022; Friehs et al., 2022; Perrey, 2023), working memory (Röhner et al., 2018; Zaehle et al., 2011), and perception (He et al., 2022; Wang et al., 2020) in healthy individuals. However, the efficacy and reliability of these interventions in human participants is often constrained by the intensity of stimulation that can be comfortably administered. As

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the intensity increases, participants report sensations that evolve from a mere tingling to pronounced discomfort such as prickling sensations and with sufficient intensity even burning or pain sensations (Fertonani et al., 2015; Hsu et al., 2021; Khadka et al., 2020; Kuhn et al., 2010; McFadden et al., 2011; Palmer et al., 1999; Paneri et al., 2016; Zeng et al., 2019).

Consequently, tES studies in humans usually stay at or below a stimulation intensity of 2 mA (Antal & Paulus, 2013; Bikson et al., 2009), with only very few studies employing higher intensities of up to 4 mA (e.g. Chhatbar et al., 2017; Hsu et al., 2023), albeit this being still a safe stimulation intensity (Antal et al., 2017; Bikson et al., 2016; Chhatbar et al., 2017; Matsumoto & Ugawa, 2017; Nitsche & Bikson, 2017). This may be due to participant compliance issues caused by uncomfortable cutaneous sensations. Sensitivity to stimulation varies based on the method of electrostimulation and specific parameters of stimulation (Ambrus et al., 2010; Fertonani et al., 2015) such as electrode size (Kuhn et al., 2010; Turi et al., 2014) or waveform of stimulation (Baker et al., 1988; Hsu et al., 2021). There is an ongoing debate about the role that current density plays in perceivability. On one hand, larger electrodes result in a lower current density, which means less current impacts each somatosensory receptor, potentially reducing perceivability (Alon et al., 1994; Verhoeven & van Dijk, 2006). On the other hand, larger electrodes cover a greater area, leading to a spatial summation effect—namely, the recruitment of more somatosensory receptors to fire, thereby enhancing perceivability (Higashiyama & Tashiro, 1990; Nielsen & Arendt-Nielsen, 1997). Further, the stimulation duration influences perceptibility due to adaptation processes attenuating sensations. This was, for example, leveraged by Khadka et al. (2020) by gradually increasing intensity over the course of the stimulation in an adaptive procedure, enhancing participant comfort at higher stimulation intensities. When using alternating currents, another key parameter in perceptibility is frequency. Frequencies below 100 Hz, commonly used in transcranial alternating current stimulation (tACS), are more perceptible than higher frequencies (Hsu et al., 2021; Turi et al., 2013; Ward & Robertson, 1998; Zeng et al., 2019) due to the spectral specificity of neurons limiting responsiveness to high frequencies (Anderson & Munson, 1951; Hawkes & Warm, 1960; Hutcheon & Yarom, 2000; Palmer et al., 1999).

A recent advancement in the area of tES research is transcranial temporal interference stimulation (tTIS). Its efficacy in modulating brain activity has been demonstrated in animal studies, highlighting it as a promising new electrostimulation method (Acerbo et al., 2022; Carmona-Barrón et al., 2023; Grossman et al., 2017; Liu et al., 2023; Missey et al., 2021; Song et al., 2021a, 2021b; Sunshine et al., 2021; Zhang et al., 2022). TTIS stands out among other tES methods due to its enhanced stimulation depth. This is achieved through the utilization of two high-frequency alternating currents ($\geq$ 500 Hz), creating an amplitude-modulated signal at the intersection of the two fields (Grossman et al., 2017; Karimi et al., 2019; Mirzakhalili et al., 2020; Song, 2019). This signal is believed to lead to modulation of neuronal activity via entrainment effects. By carefully configuring electrode placements and adjusting the current ratios of the fields, the point of interference—and consequently, the stimulation focus—can be directed deeper into brain regions. This offers the potential to non-invasively achieve neuronal modulation in deep brain regions. However, evidence supporting tTIS's efficacy in humans remains limited, with some studies even casting doubt on its feasibility (Budde et al., 2023; von Conta et al., 2022; Iszak et al., 2023). A network modeling study by Negahbani et al. (2018) as well as single neuron modeling studies (Mirzakhalili et al., 2020; Wang et al., 2023) and an in vitro study by Esmaeilpour et al. (2021) indicate that higher tTIS intensities compared with tACS are needed to achieve similar neuronal modulation. However, other studies argue that the efficacy of tTIS is largely based on network mechanisms (Cao, 2018; Martinez et al., 2023) as well as a gradual depolarization of neurons over time (Cao et al., 2020). In this context, tTIS offers a significant advantage due to its high-frequency stimulation, which makes the stimulation intensities less perceptible, allowing the application of higher intensities without discomfort. This leads to a growing interest in increasing the stimulation intensity to fully leverage the potential of tTIS, positioning it as a promising method for both treatment and research in non-invasive deep brain stimulation (Grossman et al., 2018).

Our study aimed to establish tolerable stimulation intensity ranges for both low-frequency (10, 20 Hz) and high-frequency (500, 1000, 2000 Hz) alternating currents. We selected low frequencies due to their common use in tACS studies and high frequencies for their relevance in tTIS research. By measuring pain thresholds at these frequencies, we sought to provide a reference for determining safe and tolerable maximum stimulation intensities for future tTIS and tACS research. Similarly, with perception thresholds, our goal was to identify sub-perception intensity levels crucial for ensuring effective blinding.

Studies have advocated for the use of anesthetizing skin creams in tES studies (Antal et al., 2017; Guleyupoglu et al., 2014; Liu et al., 2018; McFadden et al., 2011), offering multiple advantages. Foremost, these creams allow for the application of higher stimulation intensities than typically feasible (McFadden et al., 2011). The anesthetized skin diminishes pain perceptions, enhancing participant comfort even at higher stimulation levels. Another significant benefit is the improved blinding of participants. Many studies compare verum (true) stimulation with sham (false/placebo/control) stimulation, where sham stimulation involves applying electrical currents that mimic the cutaneous sensations

(such as tingling or itching) of verum stimulation but differ in key aspects. These differences can include being turned off after a brief period, utilizing a different frequency, or targeting a different region. The purpose of sham stimulation, as opposed to not applying any stimulation at all, is to create a condition that feels similar to the verum condition for participants, making it harder for them to differentiate between the two types of stimulation and maintain participant blinding. Using anesthetization as a complementary approach allows to reduce perceptibility of the verum stimulation, thus facilitating participant blinding (Sheffield et al., 2022). A third advantage is the possibility to control for somatosensory perception as a confounding factor in interpretation of stimulation results. Studies have demonstrated that changes in brain activity can be achieved by somatosensory entrainment due to cutaneous sensations of stimulation (Asamoah et al., 2019; Spooner et al., 2022). Thus, recent studies have begun to control for cutaneous sensations to eliminate somatosensory entrainment as a possible confounding factor (Koganemaru et al., 2020; Turi et al., 2020).

Consequently, the second aim of our study was to investigate the influence of a topical anesthetic skin cream by quantifying its impact on somatosensory perception. This was measured by observing changes in the perception and pain thresholds resulting from the application of the anesthetization. Specifically, we aimed to evaluate the anesthetization's efficacy across various stimulation frequencies, to further support it as a future tool in studies to reduce participants' awareness of stimulation conditions and increase the limits of comfortable stimulation intensities.

Methods

Participants

We recruited 37 participants (12 male, 25 female, mean age = 23.6 years, SD = 3.93 years, range = 18–36 years) for this study. Participants with a history of epileptic seizures, psychiatric or neurological disorders, metal or electric implants in the head, or those on medication affecting the central nervous system were excluded. Prior to the experiment, all participants were briefed about the procedure, potential risks of electrostimulation, and provided written informed consent. The study received approval from the University Clinic of Magdeburg's local ethics committee and adhered to the Declaration of Helsinki guidelines.

Experimental Design

We developed the experimental paradigm using MATLAB (Version 2020a, The MathWorks Inc., Natick, MA, USA) and the Psychtoolbox 3 (Kleiner et al., 2007).

Prior to the experiment, topical skin anesthetization cream with 25 mg/g lidocaine and 25 mg/g prilocaine (Anesderm, Pierre Fabre Dermo-Kosmetic GmbH) was applied to one side of each participant's head, while the opposite side remained untreated, serving as a non-anesthetized control. The side of anesthetization was counterbalanced among participants. The anesthetizing cream remained in place for 15 min to ensure its full effect. Afterwards, it was removed with a dry tissue to be replaced by an electrically conductive gel for the following stimulation.

The experiment comprised two task blocks: the somatosensory perception threshold block and the pain threshold block, with their order counterbalanced among participants to mitigate effects of task order. In the somatosensory perception block, participants were tasked to indicate if they experienced any cutaneous sensations such as tingling or itching during stimulation. During the pain threshold block, participants had to indicate if the stimulation had induced pain in the form of stinging or burning sensations. We emphasize that the staircase procedure only gradually increased stimulation intensity over trials and decreased if pain was reported. This ensured that participants only ever experienced mild pain sensations.

A block was comprised of 10 conditions: Anesthetization (yes/no) by Frequency (10 Hz, 20 Hz, 500 Hz, 1000 Hz, 2000 Hz). While the frequency variable was operationalized through the frequency of the applied alternating current, anesthetization was operationalized based on the location of stimulation—whether stimulation was administered to the anesthetized side of a participant's head or the untreated side. Each condition had its own staircase, resulting in ten individual staircases during a block. We adopted a random interleaved staircase design, wherein each consecutive stimulation was based on a randomly selected condition (see Fig. 1A). This design was implemented specifically to minimize habituation effects, which can occur when sensitivity to stimulation decreases due to the same condition being presented consecutively. By ensuring a varied sequence of frequencies and intensities, we aimed to maintain participant sensitivity and mitigate diminishing responses to the stimulation. The total number of trials needed for a staircase to conclude, depended on how many trials were needed to determine a threshold, i.e., to fulfill one of the conclusion criteria (see "Staircase Procedure" section). After a staircase was concluded, its condition was not presented again. A block ended, when all 10 staircases were concluded.

The starting amplitudes for each condition's staircase varied based on the frequency, aligning with the premise that lower frequencies are generally more perceivable, necessitating it to begin at low intensities. Conversely, higher frequencies are less perceivable, warranting a start at higher intensities to avoid the need for presentation of many unperceivable trials before reaching an intensity level relevant for those

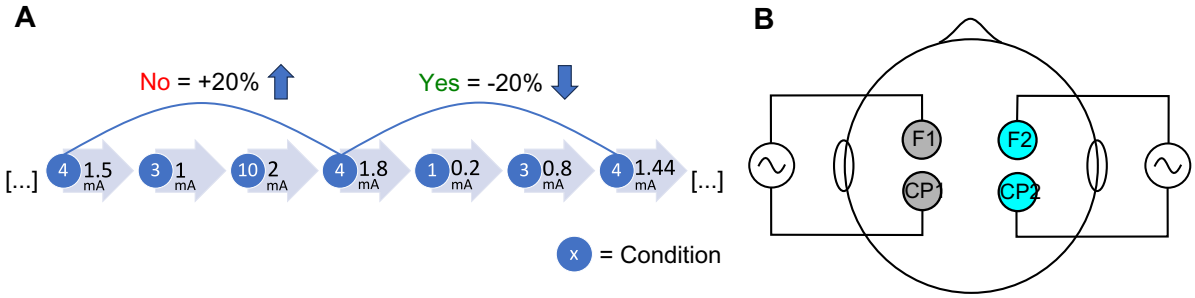

Fig. 1 **A** Consecutive trials exemplified. This illustrates how the stimulation intensity for condition 4 (1000 Hz, not anesthetized) changed depending on the subject's answer. After each trial, a question was posed to the subject (Perception block: "Did you feel the stimulation?"; Pain block: "Did you experience pain during the stimulation?"). Indicating a "No" via button press led to an increase in intensity for future presentations of that condition, whereas a "Yes" decreased the intensity. **B** Electrode montage. In this example, blue electrodes represent the side where anesthetization was applied, while grey electrodes indicate the untreated side. The side on which anesthetization was administered varied, being counterbalanced across participants

frequencies. The starting amplitudes for the conditions were as follows: 10 and 20 Hz: 0.2 mA, 500 Hz: 1 mA, 1000 Hz: 1.5 mA, 2000 Hz: 2 mA.

A trial was comprised of a 3-s countdown which was displayed on a screen in front of the participant, followed by 7 s of stimulation and ended with a self-timed period where participants had to indicate via button press if they felt the stimulation (perception threshold block) or felt pain during stimulation (pain threshold block).

Upon concluding the experiment, participants completed a questionnaire regarding potential side effects, such as lasting pain or headaches (Brunoni et al. 2011). They were then debriefed about the study's objectives and compensated with either course credit or monetarily.

Staircase Procedure

We employed an adaptive 1-up-1-down staircase procedure to determine thresholds (Cornsweet, 1962; Leek, 2001). This method estimates the stimulation strength at which participants would perceive the stimulation (perception threshold) or experience pain (pain threshold) in 50% of trials, by dynamically adjusting stimulation intensities. For example, at the end of a trial, if participants answered with "yes" to the post-stimulation question ("did you feel the stimulation" or "did you experience pain during the stimulation"), the intensity for the future presentation of that condition was decreased by 20%, based on the last given intensity. Conversely, a "no" lead to a 20% increase of intensity. A significant benefit of adaptive staircase procedures is their ability to ensure a high sampling density at and around the most relevant stimulation intensity. This approach prioritizes sampling near the intensity levels where a reversal of answers occurs, while avoiding unnecessary presentation of intensities which are far from the relevant range.

A condition's staircase could conclude in either of the following ways:

(1) After a total of five reversals of "yes/no" responses in a condition. Reversals did not have to be consecutive. The threshold was determined by averaging the last three alternating values in that staircase. To ensure a sufficient number of trials and data collection for accurately pinpointing the thresholds, we chose to require five reversals. We opted to average only the last three reversals because the initial reversals are usually further from the true threshold. In contrast, later reversals tend to be closer, making them more indicative of the actual thresholds (Leek, 2001).
(2) If a condition was presented with a stimulation intensity of 4 mA and received a "no" answer for the third time. Note that "no" answers did not have to be consecutively but were counted over the whole block. This suggests that the actual threshold for inducing sensation or pain in that condition lies above our upper limit of 4 mA. For the purposes of data analysis, we treated these instances as having a threshold of 4 mA, acknowledging that this represents the maximum intensity tested and not the actual somatosensory perception or pain threshold.
(3) If a condition has been presented for the 20th time during that block. For data analysis, the threshold was assumed as the last three alternating values.

Electrical Stimulation

Stimulation was delivered using two independent battery-driven neuroConn Stimulator systems (Advanced DC-Stimulator Plus for temporal interference stimulation, neuroConn GmbH, Ilmenau, Germany) which were connected via a digital to analog converter (Ni USB-6212, National

Instruments, Austin, TX, USA) to a PC and controlled by it using the remote mode of the stimulators. A custom MATLAB script (Version 2020a, The MathWorks Inc., Natick, MA, USA) was used to generate and send the stimulation signal. For stimulation, Ag–AgCl electrodes with a 12-mm diameter (Brain Products, Gilching, Germany) were used, which were affixed to an EEG cap (Easycap, Brain Products, Gilching, Germany). Using the international 10–10 system, electrodes were placed at positions F1 and CP1 for the left stimulation site and at F2 and CP2 for the right stimulation site (see Fig. 1 B). To increase electrode to skin conductivity, we applied a conductive paste (Abralyt 2000 abrasive electrolyte-gel, Brain Products, Gilching, Germany), ensuring impedances remained below 5 kΩ.

Stimulation frequencies were 10 Hz, 20 Hz, 500 Hz, 1000 Hz, and 2000 Hz with a maximum possible intensity of 4 mA. Our rationale for these was as follows: the 2000 Hz and 1000 Hz frequencies were used by Grossman et al. (2017) in their tTIS study and thus reflect proven and efficacious stimulation frequencies. The 500 Hz stimulation frequency is considered to be the lowest frequency feasible for tTIS (Grossman et al., 2017). Additionally, we selected 10 and 20 Hz, standard tACS stimulation frequencies prevalent in many tACS studies, to serve as a reference point to benchmark perception differences between low- and high-frequency stimulation.

Data Analysis

We performed our statistical analysis using Jamovi version 2.3 (The Jamovi Project 2024). To analyze differences between thresholds, we conducted repeated-measures analyses of variance (rmANOVAs) separately for perception- and pain thresholds using within-subject factors Anesthetization (Yes, No) and Frequency (10 Hz, 20 Hz, 500 Hz, 1000 Hz, and 2000 Hz). To ensure that factors in our rmANOVAs conformed to the sphericity assumption, we conducted a Mauchly's test.

For rmANOVA results, we report partial eta squared (η^2_p) to focus on effect size within our chosen design, as well as generalized eta squared (η^2_G) to facilitate comparing effect sizes across studies.

Results

The descriptive outcomes for perception and pain thresholds are illustrated in Fig. 2 and detailed in Table 1. In addition, Table 1 also presents the intensity values converted to current density, based on the 12 mm electrodes employed in our study.

Statistical analysis revealed violations of the sphericity assumption for perception thresholds [*Frequency*: $\chi 2\ (9) = 166.67,\ p < 0.001,\ \varepsilon = 0.49$; *Frequency * Anesthetization*: $\chi 2(9) = 132.29, p < 0.001, \varepsilon = 0.59$] as well as for pain thresholds [*Frequency*: $\chi 2(9) = 105.19, p < 0.001, \varepsilon = 0.47$; *Frequency * Anesthetization*: $\chi 2(9) = 47.96, p < 0.001, \varepsilon = 0.66$]. Given these violations, we adjusted the degrees of freedom using the Greenhouse–Geisser correction to make the test more conservative and control for type I errors.

Results of the rmANOVAs revealed a significant main effect of *Frequency* on perception- [$F(1.95,70.09) = 479.16$, $p < 0.001$, $\eta 2_p = 0.930$, $\eta 2_G = 0.796$] and pain thresholds [$F(1.86,67.11) = 588.70$, $p < 0.001$, $\eta 2_p = 0.942$, $\eta 2_G = 0.844$]. This indicates that higher frequencies lead to increased thresholds for perception and pain, implying that higher stimulation frequencies induce less cutaneous sensations than lower frequencies. This was confirmed using post-hoc analyses: as frequencies increased, so did the thresholds for both perception and pain. This was demonstrated by significant increases across all frequency comparisons for perception [all comparisons $t(36) > 3.22$, $p_{tukey} < 0.001$] and pain thresholds [all comparisons $t(36) > 5.32$, $p_{tukey} < 0.001$], with the sole exception being the pain thresholds between 10 and 20 Hz frequencies, which did not differ significantly [$t(36) = 2.26$, $p_{tukey} = 0.181$]. Additionally, a significant main effect of *Anesthetization* on thresholds was revealed, again for both perception [$F(1,36) = 19.90$, $p < 0.001$, $\eta 2_p = 0.356$, $\eta 2_G = 0.086$] and pain thresholds [$F(1,36) = 26.16$, $p < 0.001$, $\eta 2_p = 0.421$, $\eta 2_G = 0.077$], indicating that the anesthetization reduced cutaneous sensations to the stimulation in both measures. Additionally, an interaction *Frequency * Anesthetization* was observed for both perception [$F(2.36,84.85) = 6.08$, $p = 0.002$, $\eta 2_p = 0.144$, $\eta 2_G = 0.031$] and pain thresholds [$F(2.65,95.31) = 5.96$, $p = 0.001$, $\eta 2_p = 0.142$, $\eta 2_G = 0.023$]. This was due to the efficacy of anesthetization varying based on the stimulation frequency, with higher stimulation frequencies (500 and 1000 Hz) benefitting more from anesthetization than lower frequencies (10 and 20 Hz). At 2000 Hz, however, our analysis found no difference in thresholds between anesthetized and non-anesthetized conditions [perception: $t(36) = 1.47$, $p_{tukey} = 0.894$; pain: $t(36) = 1.09$, $p_{tukey} = 0.983$]. It is important to note that this absence of an anesthetization effect at 2000 Hz is a result of many subjects reaching our study's maximum stimulation of 4 mA in both anesthetized and non-anesthetized conditions, reflecting a limitation of our study setup.

Exploratory, to mitigate potential confounding effects of task order, we repeated the previous rmANOVAs including *Task Order* (pain task first, perception task first) as a between-subject factor. The results indicated that *Task Order* did not significantly affect perception thresholds [$F(1,35) = 0.225$, $p = 0.638$,$\eta 2_p = 0.006$, $\eta 2_G = 0.002$] or pain thresholds [$F(1,35) = 0.015$, $p = 0.902$, $\eta 2_p = 0.010$, $\eta 2_G = 0.004$]. Furthermore, to analyze a potential difference

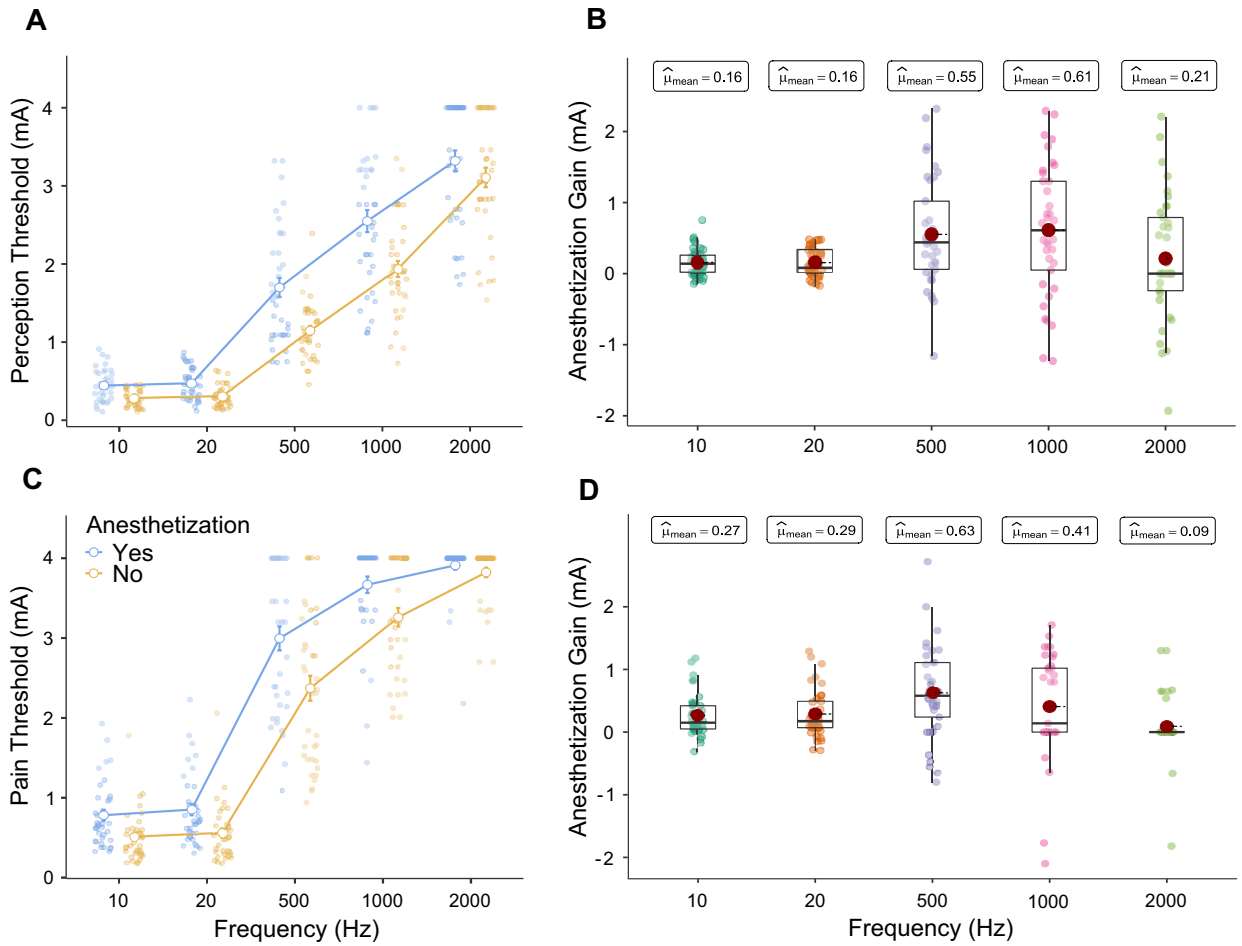

Fig. 2 Stimulation intensity thresholds. **A** Perception thresholds increase with stimulation frequency, indicating that high-frequency stimulation induces less perception. Conditions with topical anesthetization display higher thresholds. **B** Anesthetization allows for higher stimulation intensities before reaching perception thresholds across all frequencies, as indicated by *Anesthetization Gains* ($=\text{Threshold}_{\text{WithAnesthetization}} - \text{Threshold}_{\text{WithoutAnesthetization}}$). Notably, the reduced anesthetization gain at 2000 Hz is due to some participants not perceiving any stimulation at high frequencies, both with and without anesthetization. For analysis, their threshold was standardized to 4 mA, aligning anesthetized and non-anesthetized conditions and reducing observed anesthetization gains. This reflects study constraints more than a decrease in anesthetization effectiveness at higher frequencies. **(C)** Pain thresholds: Higher stimulation frequencies correlate with lower pain thresholds, reflecting the pattern seen in perception thresholds. **(D)** Anesthetization gains for pain thresholds: These gains follow a similar trend to perception thresholds, with the same high-frequency constraints previously noted

between sexes, we repeated the rmANOVAs again with *Sex* (male, female) as a between-subject factor. This analysis revealed a significant effect of *Sex* on pain thresholds [$F(1,35)=4.34$, $p=0.045$, $\eta 2_p=0.110$, $\eta 2_G=0.048$]. Additionally, a significant interaction effect *Frequency* * *Sex* was observed [$F(1.99,69.50)=5.346$, $p=0.007$, $\eta 2_p=0.133$, $\eta 2_G=0.046$], which is descriptively explained by male participants being able to tolerate higher intensities at high frequencies, though this did not reach significance in post-hoc analysis [male vs. female; 10 Hz: *Mean Diff* = 0.01 mA, $t(35)=0.085$, $p_{tukey}=1.000$; 20 Hz: *Mean Diff* = 0.08 mA, $t(35)=0.665$, $p_{tukey}=1.000$; 500 Hz: *Mean Diff* = 0.71 mA, $t(35)=0.665$, $p_{tukey}=0.250$; 1000 Hz: *Mean Diff* = 0.42 mA, $t(35)=2.413$, $p_{tukey}=0.349$; 2000 Hz: *Mean Diff* = 0.12 mA, $t(35)=1.322$, $p_{tukey}=0.942$]. No other effects reached significance. Further, no significant effect of *Sex* on perception thresholds [$F(1,35)=0.097$, $p=0.758$, $\eta 2_p=0.003$., $\eta 2_G=0.001$] could be observed.

Discussion

Current human applications of tES are limited by the maximum intensity that participants can comfortably tolerate. Even though higher intensities are considered safe (Antal et al., 2017; Bikson et al., 2016; Chhatbar et al., 2017; Matsumoto & Ugawa, 2017), the discomfort from skin sensations or pain often restricts their use. The recent introduction of tTIS (Grossman et al., 2017) leverages the fact that high-frequency alternating currents are

Table 1 Descriptive statistics of perception and pain thresholds

	Without anesthetization					With anesthetization				
	10 Hz	20 Hz	500 Hz	1000 Hz	2000 Hz	10 Hz	20 Hz	500 Hz	1000 Hz	2000 Hz
Perception thresholds										
Mean (mA)	0.29	0.31	1.15	1.94	3.11	0.44	0.47	1.70	2.55	3.32
Mean CD (mA/cm^2)	0.26	0.27	1.02	1.72	2.75	0.39	0.42	1.50	2.25	2.94
Median (mA)	0.27	0.28	1.13	1.90	3.07	0.43	0.46	1.58	2.53	4.00
SD (mA)	0.11	0.14	0.38	0.62	0.76	0.21	0.20	0.74	0.86	0.81
Min (mA)	0.11	0.13	0.46	0.73	1.54	0.11	0.12	0.74	1.12	1.74
Max (mA)	0.46	0.64	2.39	3.35	4.00	0.91	0.87	3.32	4.00	4.00
N_{Lim}/N_{Total}	0/37	0/37	0/37	0/37	11/37	0/37	0/37	0/37	4/37	19/37
Pain thresholds										
Mean (mA)	0.51	0.56	2.37	3.26	3.82	0.78	0.85	3.00	3.67	3.91
Mean CD (mA/cm^2)	0.45	0.50	2.10	2.88	3.38	0.69	0.75	2.65	3.25	3.46
Median (mA)	0.44	0.52	2.35	3.21	4.00	0.67	0.71	3.20	4.00	4.00
SD (mA)	0.31	0.35	0.96	0.71	0.37	0.40	0.44	0.92	0.63	0.34
Min (mA)	0.18	0.18	0.94	2.01	2.70	0.33	0.31	1.09	1.44	2.18
Max (mA)	1.78	2.06	4.00	4.00	4.00	1.93	2.23	4.00	4.00	4.00
N_{Lim}/N_{Total}	0/37	0/37	4/37	15/37	29/37	0/37	0/37	13/37	27/37	34/37

N_{Lim}, number of participants reaching the maximum stimulation intensity threshold (4 mA) in that condition; N_{Total}, total number of participants; *CD*, current density

less perceivable (Hsu et al., 2021; Hutcheon & Yarom, 2000; Turi et al., 2013; Zeng et al., 2019), thus potentially allowing for the application of higher stimulation intensities. In our study, we sought to quantify the sensation and pain thresholds for various high-frequency alternating current stimulations used in tTIS, aiming to explore the potential upper limits of intensity for this innovative electrostimulation technique. Furthermore, we explored the possibility to push these limits by employing an anesthetizing topical cream, to reduce cutaneous sensations caused by electrical stimulation. To be able benchmark the perception of these high frequencies against classical methods of electrostimulation, we've also quantified perception of low-frequency alternating currents as commonly used in tACS.

Our findings are in line with other studies, demonstrating that as stimulation frequency increases, its perceptibility decreases (Hsu et al., 2021; Imatz-Ojanguren & Keller, 2023; Turi et al., 2013; Ward & Robertson, 1998; Zeng et al., 2019). Additionally, consistent with findings from other studies, we verified that application of an anesthetizing cream reduces perceptibility during transcranial electrostimulation (Guleyupoglu et al., 2014; McFadden et al., 2011). The reduced perceptibility of high-frequency stimulation offers an added advantage for ensuring participant blinding, given its increased perception thresholds compared to traditional electrostimulation methods. Moreover, the use of an anesthetizing cream appears to be a valuable tool in increasing participant comfort and blinding.

The included lower frequencies of 10 Hz and 20 Hz in our study reflect standard frequencies employed in tACS studies (Herrmann & Strüber, 2017; Koninck et al., 2023; Wischnewski et al., 2019; Yavari et al., 2018). Our findings demonstrate that these frequencies are already perceptible for most participants at low stimulation intensities, while discomfort or pain became noticeable at slightly higher intensities. In addition, the application of the anesthetizing cream was able to increase these thresholds, proving its effectiveness in reducing somatosensory side effects during stimulation like tingling, itching, or stinging sensations. Furthermore, the higher frequencies of 500 Hz, 1000 Hz, and 2000 Hz selected for this study reflect stimulation frequencies used in current tTIS studies (von Conta et al., 2022; Esmaeilpour et al., 2021; Grossman et al., 2017; Ma et al., 2021; Sunshine et al., 2021; Zhu et al., 2022). Due to reduced sensitivity to higher frequencies in somatosensory perception (Hutcheon & Yarom, 2000; Palmer et al., 1999), we were able to confirm that these frequencies allow for a substantial increase in stimulation intensity before becoming perceptible or inducing pain. In addition, we were able to push the limits of comfortable stimulation intensities even further with the use of anesthetization. Notably, our results indicate that the anesthetic effect seemed to decrease at frequencies of 1000 Hz and above. However, as explained, this is not indicative of reduced anesthetic efficacy, but rather a ceiling effect inherent to our study design. Nonetheless, these results highlight the potential for using high-frequency stimulation in conjunction with topical anesthetization at

increased intensities, without compromising participant blinding or causing discomfort.

Notably, for the higher frequencies, a portion of the participants had their thresholds set to 4 mA for statistical analysis in line with staircase conclusion criterion 2. This indicates that even when the maximum stimulation intensity of 4 mA was reached in a condition, these participants did not report pain or perceivable sensations. This is especially true for stimulation with 2000 Hz, in which 29 out of 37 subjects reached 4 mA. Therefore, it's crucial to recognize that the actual thresholds for these frequencies likely surpass our applied maximum of 4 mA. This limitation is due to the ethical, safety, and hardware constraints within which our study was conducted. Consequently, the recorded thresholds at these frequencies essentially represent the highest stimulation intensities we could safely administer, highlighting that participants might have tolerated even higher intensities during high-frequency stimulation without discomfort. Therefore, our statistical analysis is even on the conservative side and likely underestimates the true tolerable thresholds.

On the other side, some subjects reported pain sensations for high frequencies at considerably lower intensities (e.g., for 2000 Hz, one participant's pain threshold was measured as 2.7 mA). This highlights the interindividual differences in perception to electrostimulation which should be taken into account. Especially for low frequencies, our results reveal considerable variability in pain and perception thresholds among participants. For instance, in the 10 Hz condition, pain thresholds range from 0.18 to 1.78 mA. This highlights that both high- and low-frequency stimulation vary in individual sensitivity and there is no one-size-fits-all approach. Therefore, our thresholds should be viewed as approximate guidelines rather than absolute values. However, our data indicate that even in common tACS studies, it is highly probable that some subjects will perceive the stimulation and few subjects will even feel pain, even if the stimulation is within a safe range of <2 mA. Thus, for optimal safety and successful blinding, we advise testing individual thresholds before applying electrostimulation at the target intensity.

It is essential to recognize that the effectiveness of stimulation is not solely determined by the applied stimulation intensity. While numerous studies suggest a dose-dependent effect, indicating that higher stimulation intensities often lead to more pronounced effects (Johnson et al., 2020; Turner et al., 2021; Wischnewski et al., 2019), this is not an absolute rule. Indeed, some research indicates a complex, non-linear relationship between stimulation intensity and its effects. A study by Moliadze et al. (2012) has shown that while lower intensities might lead to inhibition, increasing the intensity can actually reverse this effect, transforming inhibition into excitation. Moreover, the actual voltage that reaches the target area is not guaranteed by high stimulation intensity alone. Variabilities in individual anatomical factors, such as skull thickness, and the specific configuration of the electrode montage, significantly influence the voltage delivered to the target area (Hunold et al., 2023). Consequently, it is recommended to utilize current flow modeling tools, like SimNIBS (Puonti et al., 2020; Thielscher et al., 2015) or ROAST (Huang et al., 2019). These tools, particularly when modeled on individual anatomical specifics, can be used to ensure that the target area receives sufficient voltage for effective stimulation (Saturnino et al., 2019).

It is important to emphasize that the stimulation thresholds in our study were determined using round electrodes with a diameter of 12 mm. To ensure that our results are relevant irrespective of electrode size, we have included measures of current density alongside our findings. However, it's essential to understand the interplay between electrode size, current density, and current intensity. Several studies have posited that larger electrodes generally offer more comfort than smaller ones, attributed to the distribution of currents across a larger area, leading to lower current densities (Alon et al., 1994; McNeal & Baker, 1988; Verhoeven & van Dijk, 2006). Yet, Lyons et al. (2004) presented contrasting evidence, showing greater comfort with smaller electrodes. This contradiction may be solved by recent research, which suggests that cutaneous sensation is primarily influenced by current intensity rather than current density (Fertonani et al., 2015; Martinsen et al., 2004; Turi et al., 2014). This is due to a spatial summation effect where larger electrodes engage more cutaneous receptors, increasing sensation (Higashiyama, 1993; Higashiyama & Tashiro, 1990; Nielsen & Arendt-Nielsen, 1997). Consequently, our results regarding perception and pain thresholds still provide valuable guidance for studies using larger electrodes. However, it's worth noting that cutaneous sensations can be influenced by other variables, such as the concentration of a saline solution used as a contact medium (Dundas et al., 2007), though they are not affected by the shape of the electrode (Ambrus et al., 2011).

Certainly, safe stimulation intensities cannot be based solely on the lack of immediate cutaneous pain sensations. Research confirms the safety of classical electrostimulation, as evidenced by rodent model studies. These studies show no changes in several neurotoxicity markers for stimulation intensities commonly used in humans (Jackson et al., 2017; Liebetanz et al., 2009; Zhang et al., 2019). This becomes also evident in human studies, which confirm the absence of neurotoxicity or serious adverse effects of stimulation (Nitsche et al., 2003; Nitsche et al., 2004; 2001; Tadini et al., 2011). Currently, stimulation intensities of up to 4 mA are considered safe using electrostimulation methods such as tACS or tDCS (Antal et al., 2017; Bikson et al., 2016; Fertonani et al., 2015; Matsumoto & Ugawa, 2017; Nitsche & Bikson, 2017). Taking into account the novel high-frequency stimulation methods, Grossman et al. (2017) did not find tissue damage in rodents,

and Piao et al. (2022) did not find an adverse effect of this stimulation on various tested criteria in humans. This is in line with Cassarà et al. (2022), who explored the safety of tTIS in humans and recommended frequency-based maximum exposure limits, with higher frequencies allowing for greater exposure. Nonetheless, to increase stimulation intensities above 4 mA, a robust body of evidence pointing to its unquestionable safety is needed.

A limitation of our findings is the brief 5-s duration of stimulation to measure thresholds. Previous research indicates that stimulation sensation decreases over time due to adaptation effects (Hsu et al., 2021; Khadka et al., 2020). This adaptation effect explains why tACS studies can administer intensities of 1 mA or more without causing prolonged discomfort. Consequently, the thresholds identified in our study are likely conservative, potentially underestimating the maximum tolerable stimulation intensities. Employing a procedure where stimulation is applied for an extended duration with a gradual increase could leverage this adaptation effect to even further increase perception and pain thresholds. This would allow for even more intense, yet still comfortable, electrical stimulation. Future research should explore the limits of this adaptive approach for high-frequency alternating current stimulation.

In addition, in our study, we relied on participants' self-reports to determine thresholds, a method that inherently carries the risk of subjective biases. To enhance the robustness of future studies, incorporating objective indicators, like physiological markers of discomfort including skin conductivity (Storm, 2008; Syrjala et al., 2019), might offer a more consistent gauge of participant comfort.

Furthermore, is well-documented that pain perception can be influenced by an individual's physical and psychological state. Factors such as age (Lautenbacher et al., 2017), expectation (Wiech, 2016; Wiech et al., 2008), fatigue (Lautenbacher et al., 2006), or sex (Paller et al., 2009; Wiesenfeld-Hallin, 2005) influence how pain is experienced. This is in line with our study's results, where males were able to tolerate higher stimulation intensities compared with females. However, given that the sample in our study had a bias towards female participants (25 female, 12 male), the generalization of our findings should be done with a degree of caution. A future study systematically comparing sex differences would be needed to substantiate this result. However, we believe that our results serve as a robust foundation for establishing new limits and possibilities for future stimulation studies.

Conclusion

We demonstrated that the somatosensory perception and pain thresholds for alternating current stimulation are frequency-dependent. Utilizing high-frequency stimulation, we successfully administered intensities of up to 4 mA without inducing discomfort in participants. This finding is especially of note for tTIS, whose efficacy has been limited by low-intensity protocols so far. Increasing the stimulation intensity has the potential to enhance the efficacy of tTIS, unlocking the potential for non-invasive stimulation of deeper brain regions. Additionally, the use of topical anesthetic cream further elevates these thresholds, enabling even higher intensities. This finding also translates to tACS applications in general, allowing for more potent neuromodulatory interventions without compromising participant comfort.

In summary, our findings reveal significant interindividual differences in perception and pain thresholds, particularly under high-frequency conditions, emphasizing the need for customized stimulation intensities in tTIS/tACS experiments. To ensure participant comfort and effective blinding, we recommend tailoring stimulation based on individual responses. Additionally, our study shows that using a topical anesthetic can raise these thresholds, offering a viable method to enhance participant tolerance or blinding in future electrostimulation studies.

AI and AI-Assisted Technologies in the Writing Process

During the preparation of this work, the authors used ChatGPT-4 in order to improve readability and language of the manuscript. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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Author Contribution Carsten Thiele: Conceptualization; methodology; software; investigation; formal analysis; data curation; writing, original draft; visualization.

Cornelius Tamm: Investigation, formal analysis, writing—review and editing.

Philipp Ruhnau: Resources; writing, review and editing; supervision; project administration; funding acquisition.

Tino Zaehle: Conceptualization; resources; writing, review and editing; supervision; project administration; funding acquisition.

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Data Availability The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of Interest The authors declare no competing interests.

References

Acerbo, E., Jegou, A., Luff, C., Dzialecka, P., Botzanowski, B., Missey, F., Ngom, I., Lagarde, S., Bartolomei, F., Cassara, A., Neufeld, E., Jirsa, V., Carron, R., Grossman, N., & Williamson, A. (2022). Focal non-invasive deep-brain stimulation with temporal interference for the suppression of epileptic biomarkers. *Frontiers in Neuroscience, 16*, 945221.

Alon, G., Kantor, G., & Ho, H. S. (1994). Effects of electrode size on basic excitatory responses and on selected stimulus parameters. *The Journal of Orthopaedic and Sports Physical Therapy, 20*(1), 29–35.

Ambrus, G. G., Paulus, W., & Antal, A. (2010). Cutaneous perception thresholds of electrical stimulation methods: Comparison of tDCS and tRNS. *Clinical Neurophysiology, 121*(11), 1908–1914.

Ambrus, G. G., Antal, A., & Paulus, W. (2011). Comparing cutaneous perception induced by electrical stimulation using rectangular and round shaped electrodes. *Clinical Neurophysiology Official Journal of the International Federation of Clinical Neurophysiology, 122*(4), 803–807.

Anderson, A. B., & Munson, W. A. (1951). Electrical excitation of nerves in the skin at audiofrequencies. *The Journal of the Acoustical Society of America, 23*(2), 155–159.

Antal, A., & Paulus, W. (2013). Transcranial alternating current stimulation (tACS). *Frontiers in Human Neuroscience, 7*, 317.

Antal, A., Alekseichuk, I., Bikson, M., Brockmöller, J., Brunoni, A. R., Chen, R., Cohen, L. G., Dowthwaite, G., Ellrich, J., Flöel, A., Fregni, F., George, M. S., Hamilton, R., Haueisen, J., Herrmann, C. S., Hummel, F. C., Lefaucheur, J. P., Liebetanz, D., Loo, C. K., … Paulus, W. (2017). Low intensity transcranial electric stimulation: Safety, ethical, legal regulatory and application guidelines. *Clinical Neurophysiology, 128*(9), 1774–1809.

Arul-Anandam, A. P., & Loo, C. (2009). Transcranial direct current stimulation: A new tool for the treatment of depression? *Journal of Affective Disorders, 117*(3), 137–145.

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

Baker, L. L., Bowman, B. R., & McNeal. (1988). Effects of waveform on comfort during neuromuscular electrical stimulation. *Clinical Orthopaedics and Related Research, 233*, 75–85.

Bikson, M., Datta, A., & Elwassif, M. (2009). Establishing safety limits for transcranial direct current stimulation. *Clinical Neurophysiology, 120*(6), 1033–1034.

Bikson, M., Grossman, P., Thomas, C., Zannou, A. L., Jiang, J., Adnan, T., Mourdoukoutas, A. P., Kronberg, G., Truong, D., Boggio, P., Brunoni, A. R., Charvet, L., Fregni, F., Fritsch, B., Gillick, B., Hamilton, R. H., Hampstead, B. M., Jankord, R., Kirton, A., … Woods, A. J. (2016). Safety of transcranial direct current stimulation: Evidence based update 2016. *Brain Stimulation, 9*(5), 641–661.

Budde, R. B., Williams, M. T., & Irazoqui. (2023). Temporal interference current stimulation in peripheral nerves is not driven by envelope extraction. *Journal of Neural Engineering, 20*(2), 026041.

Cao J, ron B, Goswami C, and Grover P. The mechanics of temporal interference stimulation. *bioRxiv*: 2020.04.23.051870, 2020.

Cao J. 2018 Do single neuron models exhibit temporal interference stimulation?, 2018 Cao J, Grover P. Do single neuron models exhibit temporal interference stimulation?. In2018 IEEE Biomedical Circuits and Systems Conference (BioCAS) 2018 Oct 17 (pp. 1-4). IEEE

Carmona-Barrón VG, Fernández del Campo IS, Delgado-García JM, De la Fuente AJ, Lopez IP, Merchán MA. 2023 Comparing the effects of transcranial alternating current and temporal interference (tTIS) electric stimulation through whole-brain mapping of c-Fos immunoreactivity. Frontiers in Neuroanatomy 17 1128193

Chang, S. (2022). The application of transcranial electrical stimulation in sports psychology. *Computational and Mathematical Methods in Medicine, 2022*, 1008346.

Chhatbar, P. Y., Chen, R., Deardorff, R., Dellenbach, B., Kautz, S. A., George, M. S., & Feng, W. (2017). Safety and tolerability of transcranial direct current stimulation to stroke patients - A phase I current escalation study. *Brain Stimulation, 10*(3), 553–559.

Cho, H., Razza, L. B., Borrione, L., Bikson, M., Charvet, L., Dennis-Tiwary, T. A., Brunoni, A. R., & Sudbrack-Oliveira, P. (2022). Transcranial electrical stimulation for psychiatric disorders in adults: A primer. *Focus, 20*(1), 19–31.

Convento, S., Russo, C., Zigiotto, L., & Bolognini, N. (2016). Transcranial electrical stimulation in post-stroke cognitive rehabilitation. *European Psychologist, 21*(1), 55–64.

Cornsweet, T. N. (1962). The staircase-method in psychophysics. *The American Journal of Psychology, 75*(3), 485–491.

De Koninck, B. P., Brazeau, D., Guay, S., Babiloni, A. H., & De Beaumont, L. (2023). Transcranial alternating current stimulation to modulate alpha activity: A systematic review. *Neuromodulation: Technology at the Neural Interface, 26*(8), 1549–84.

Dundas, J. E., Thickbroom, G. W., & Mastaglia, F. L. (2007). Perception of comfort during transcranial DC stimulation: Effect of NaCl solution concentration applied to sponge electrodes. *Clinical Neurophysiology, 118*(5), 1166–1170.

Esmaeilpour, Z., Kronberg, G., Reato, D., Parra, L. C., & Bikson, M. (2021). Temporal interference stimulation targets deep brain regions by modulating neural oscillations. *Brain Stimulation, 14*(1), 55–65.

Fertonani, A., Ferrari, C., & Miniussi, C. (2015). What do you feel if I apply transcranial electric stimulation? Safety, sensations and secondary induced effects. *Clinical Neurophysiology, 126*(11), 2181–2188.

Friehs, M. A., Whelan, E., Güldenpenning, I., Krause, D., & Weigelt, M. (2022). Stimulating performance: A scoping review on transcranial electrical stimulation effects on Olympic sports. *Psychology of Sport and Exercise, 59*, 102130.

Grossman, N., Bono, D., Dedic, N., Kodandaramaiah, S. B., Rudenko, A., Suk, H.-J., Cassara, A. M., Neufeld, E., Kuster, N., & Tsai, L.-H. (2017). Noninvasive deep brain stimulation via temporally interfering electric fields. *Cell, 169*(6), 1029–1041.

Grossman, N., Okun, M. S., & Boyden, E. S. (2018). Translating temporal interference brain stimulation to treat neurological and psychiatric conditions. *JAMA Neurology, 75*(11), 1307–1308.

Guleyupoglu, B., Febles, N., Minhas, P., Hahn, C., & Bikson, M. (2014). Reduced discomfort during high-definition

transcutaneous stimulation using 6% benzocaine. *Frontiers in Neuroengineering, 7*, 28.
Hawkes, G. R., & Warm, J. S. (1960). The sensory range of electrical stimulation of the skin. *The American Journal of Psychology, 73*(3), 485.
He, Q., Yang, X.-Y., Zhao, D., & Fang, F. (2022). Enhancement of visual perception by combining transcranial electrical stimulation and visual perceptual training. *Medical Review, 2*(3), 271–284.
Herrmann, C. S., & Strüber, D. (2017). What can transcranial alternating current stimulation tell us about brain oscillations? *Current Behavioral Neuroscience Reports, 4*(2), 128–137.
Higashiyama, A. (1993). Electrocutaneous spatial integration at suprathreshold levels: An additive neural model. *Journal of Experimental Psychology: Human Perception and Performance, 19*(4), 912–923.
Higashiyama, A., & Tashiro, T. (1990). Electrocutaneous spatial integration at threshold: The effects of electrode size. *Perception & Psychophysics, 48*(4), 389–397.
Hsu, G., Farahani, F., & Parra, L. C. (2021). Cutaneous sensation of electrical stimulation waveforms. *Brain Stimulation, 14*(3), 693–702.
Hsu, G., Shereen, A. D., Cohen, L. G., & Parra, L. C. (2023). Robust enhancement of motor sequence learning with 4 mA transcranial electric stimulation. *Brain Stimulation, 16*(1), 56–67.
Huang, Y., Datta, A., Bikson, M., & Parra, L. C. (2019). Realistic volumetric-approach to simulate transcranial electric stimulation-ROAST-A fully automated open-source pipeline. *Journal of Neural Engineering, 16*(5), 56006.
Hunold, A., Haueisen, J., Nees, F., & Moliadze, V. (2023). Review of individualized current flow modeling studies for transcranial electrical stimulation. *Journal of Neuroscience Research, 101*(4), 405–423.
Hutcheon, B., & Yarom, Y. (2000). Resonance, oscillation and the intrinsic frequency preferences of neurons. *Trends in Neurosciences, 23*(5), 216–222.
Imatz-Ojanguren, E., & Keller, T. (2023). Evoked sensations with transcutaneous electrical stimulation with different frequencies, waveforms, and electrode configurations. *Artificial Organs, 47*(1), 117–128.
Iszak, K., Gronemann, S. M., Meyer, S., Hunold, A., Zschüntzsch, J., Bähr, M., Paulus, W., & Antal, A. (2023). Why temporal inference stimulation may fail in the human brain: A pilot research study. *Biomedicines, 11*(7), 2023.
Jackson, M. P., Truong, D., Brownlow, M. L., Wagner, J. A., McKinley, R. A., Bikson, M., & Jankord, R. (2017). Safety parameter considerations of anodal transcranial direct current stimulation in rats. *Brain, Behavior, and Immunity, 64*, 152–161.
Johnson, L., Alekseichuk, I., Krieg, J., Doyle, A., Yu, Y., Vitek, J., Johnson, M., & Opitz, A. (2020). Dose-dependent effects of transcranial alternating current stimulation on spike timing in awake nonhuman primates. *Science Advances, 6*(36), eaaz2747.
Karimi, F., Attarpour, A., Amirfattahi, R., & Nezhad, A. Z. (2019). Computational analysis of non-invasive deep brain stimulation based on interfering electric fields. *Physics in Medicine and Biology, 64*(23), 235010.
Khadka, N., Borges, H., Paneri, B., Kaufman, T., Nassis, E., Zannou, A. L., Shin, Y., Choi, H., Kim, S., Lee, K., & Bikson, M. (2020). Adaptive current tDCS up to 4 mA. *Brain Stimulation, 13*(1), 69–79.
Khan, A., Yuan, K., Bao, S.-C., Ti, C. H. E., Tariq, A., Anjum, N., & Tong, R.K.-Y. (2022). Can transcranial electrical stimulation facilitate post-stroke cognitive rehabilitation? A systematic review and meta-analysis. *Frontiers in Rehabilitation Sciences, 3*, 795737.
Kleiner M, Brainard D, and Pelli D. 2007 What's new in Psychtoolbox-3?
Koganemaru, S., Mikami, Y., Matsuhashi, M., Truong, D. Q., Bikson, M., Kansaku, K., & Mima, T. (2020). Cerebellar transcranial alternating current stimulation modulates human gait rhythm. *Neuroscience Research, 156*, 265–270.
Kuhn, A., Keller, T., Lawrence, M., & Morari, M. (2010). The influence of electrode size on selectivity and comfort in transcutaneous electrical stimulation of the forearm. *IEEE Transactions on Neural Systems and Rehabilitation Engineering, 18*(3), 255.
Lautenbacher, S., Kundermann, B., & Krieg, J.-C. (2006). Sleep deprivation and pain perception. *Sleep Medicine Reviews, 10*(5), 357–369.
Lautenbacher, S., Peters, J. H., Heesen, M., Scheel, J., & Kunz, M. (2017). Age changes in pain perception: A systematic-review and meta-analysis of age effects on pain and tolerance thresholds. *Neuroscience and Biobehavioral Reviews, 75*, 104–113.
Leek, M. R. (2001). Adaptive procedures in psychophysical research. *Perception & Psychophysics, 63*(8), 1279–1292.
Liebetanz, D., Koch, R., Mayenfels, S., König, F., Paulus, W., & Nitsche, M. A. (2009). Safety limits of cathodal transcranial direct current stimulation in rats. *Clinical Neurophysiology Official Journal of the International Federation of Clinical Neurophysiology, 120*(6), 1161–1167.
Linnhoff, S., Fiene, M., Heinze, H.-J., & Zaehle, T. (2019). Cognitive fatigue in multiple sclerosis: An objective approach to diagnosis and treatment by transcranial electrical stimulation. *Brain Sciences, 9*(5), 100.
Liu, A., Vöröslakos, M., Kronberg, G., Henin, S., Krause, M. R., Huang, Y., Opitz, A., Mehta, A., Pack, C. C., Krekelberg, B., Berényi, A., Parra, L. C., Melloni, L., Devinsky, O., & Buzsáki, G. (2018). Immediate neurophysiological effects of transcranial electrical stimulation. *Nature Communications, 9*(1), 5092.
Liu, X., Qi, S., Hou, L., Liu, Y., & Wang, X. (2024). Noninvasive deep brain stimulation via temporal interference electric fields enhanced motor performance of mice and its neuroplasticity mechanisms. *Molecular Neurobiology, 61*(6), 3314–29.
Lyons, G. M., Leane, G. E., Clarke-Moloney, M., O'Brien, J. V., & Grace, P. A. (2004). An investigation of the effect of electrode size and electrode location on comfort during stimulation of the gastrocnemius muscle. *Medical Engineering & Physics, 26*(10), 873–878.
Ma, R., Xia, X., Zhang, W., Lu, Z., Wu, Q., Cui, J., Song, H., Fan, C., Chen, X., & Zha, R. (2021). High gamma and beta temporal interference stimulation in the human motor cortex improves motor functions. *Frontiers in Neuroscience, 15*, 800436.
Martinez, S. C., Goswami, C., Forssell, M., Cao, J., Barth, A., & Grover, P. (2023). Cell-specific effects of temporal interference stimulation on cortical function. *Research Square*, PREPRINT (Version 1). https://doi.org/10.21203/rs.3.rs-2395375/v1
Martinsen, Ø. G., Grimnes, S., & Piltan, H. (2004). Cutaneous perception of electrical direct current. *ITBM-RBM, 25*(4), 240–243.
Matsumoto, H., & Ugawa, Y. (2017). Adverse events of tDCS and tACS: A review. *Clinical Neurophysiology Practice, 2*, 19–25.
McFadden, J. L., Borckardt, J. J., George, M. S., & Beam, W. (2011). Reducing procedural pain and discomfort associated with transcranial direct current stimulation. *Brain Stimulation, 4*(1), 38–42.
McNeal, D. R., & Baker, L. L. (1988). Effects of joint angle, electrodes and waveform on electrical stimulation of the quadriceps and hamstrings. *Annals of Biomedical Engineering, 16*(3), 299–310.
Mirzakhalili, E., Barra, B., Capogrosso, M., & Lempka, S. F. (2020). Biophysics of temporal interference stimulation. *Cell Systems, 11*(6), 557-572.e5.
Missey, F., Rusina, E., Acerbo, E., Botzanowski, B., Trébuchon, A., Bartolomei, F., Jirsa, V., Carron, R., & Williamson, A. (2021). Orientation of temporal interference for non-invasive deep brain stimulation in epilepsy. *Frontiers in Neuroscience, 15*, 633988.

Moliadze, V., Atalay, D., Antal, A., & Paulus, W. (2012). Close to threshold transcranial electrical stimulation preferentially activates inhibitory networks before switching to excitation with higher intensities. *Brain Stimulation, 5*(4), 505–511.

Negahbani, E., Kasten, F. H., Herrmann, C. S., & Fröhlich, F. (2018). Targeting alpha-band oscillations in a cortical model with amplitude-modulated high-frequency transcranial electric stimulation. *NeuroImage, 173*, 3–12.

Nielsen, J., & Arendt-Nielsen, L. (1997). Spatial summation of heat induced pain within and between dermatomes. *Somatosensory & Motor Research, 14*(2), 119–125.

Nitsche, M. A., & Bikson, M. (2017). Extending the parameter range for tDCS: Safety and tolerability of 4 mA stimulation. *Brain Stimulation, 10*(3), 541–542.

Nitsche, M. A., & Paulus, W. (2001). Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans. *Neurology, 57*(10), 1899–1901.

Nitsche, M. A., Nitsche, M. S., Klein, C. C., Tergau, F., Rothwell, J. C., & Paulus, W. (2003). Level of action of cathodal DC polarisation induced inhibition of the human motor cortex. *Clinical Neurophysiology, 114*(4), 600–604.

Nitsche, M. A., Niehaus, L., Hoffmann, K. T., Hengst, S., Liebetanz, D., Paulus, W., & Meyer, B.-U. (2004). MRI study of human brain exposed to weak direct current stimulation of the frontal cortex. *Clinical Neurophysiology, 115*(10), 2419–2423.

Paller, C. J., Campbell, C. M., Edwards, R. R., & Dobs, A. S. (2009). Sex-based differences in pain perception and treatment. *Pain Medicine, 10*(2), 289–299.

Palmer, S. T., Martin, D. J., Steedman, W. M., & Ravey, J. (1999). Alteration of interferential current and transcutaneous electrical nerve stimulation frequency: Effects on nerve excitation. *Archives of Physical Medicine and Rehabilitation, 80*(9), 1065–1071.

Paneri, B., Adair, D., Thomas, C., Khadka, N., Patel, V., Tyler, W. J., Parra, L., & Bikson, M. (2016). Tolerability of repeated application of transcranial electrical stimulation with limited outputs to healthy subjects. *Brain Stimulation, 9*(5), 740–754.

Perrey, S. (2023). Probing the promises of noninvasive transcranial electrical stimulation for boosting mental performance in sports. *Brain Sciences, 13*(2), 282.

Piao, Y., Ma, R., Weng, Y., Fan, C., Xia, X., Zhang, W., Zeng, G. Q., Wang, Y., Lu, Z., Cui, J., Wang, X., Gao, L., Qiu, B., & Zhang, X. (2022). Safety evaluation of employing temporal interference transcranial alternating current stimulation in human studies. *Brain Sciences, 12*(9), 1194.

Puonti, O., van Leemput, K., Saturnino, G. B., Siebner, H. R., Madsen, K. H., & Thielscher, A. (2020). Accurate and robust whole-head segmentation from magnetic resonance images for individualized head modeling. *NeuroImage, 219*, 117044.

Röhner, F., Breitling, C., Rufener, K. S., Heinze, H.-J., Hinrichs, H., Krauel, K., & Sweeney-Reed, C. M. (2018). Modulation of working memory using transcranial electrical stimulation: A direct comparison between TACS and TDCS. *Frontiers in Neuroscience, 12*, 761.

Saturnino, G. B., Siebner, H. R., Thielscher, A., & Madsen, K. H. (2019). Accessibility of cortical regions to focal TES: Dependence on spatial position, safety, and practical constraints. *NeuroImage, 203*, 116183.

Sheffield, J. G., Ramerpresad, S., Brem, A.-K., Mansfield, K., Orhan, U., Dillard, M., McKanna, J., Plessow, F., Thompson, T., Santarnecchi, E., Pascual-Leone, A., Pavel, M., Mathan, S., & Cohen, K. R. (2022). Blinding efficacy and adverse events following repeated transcranial alternating current, direct current, and random noise stimulation. *Cortex, 154*, 77–88.

Shirvani, S., Davoudi, M., Shirvani, M., Koleini, P., Hojat Panah, S., Shoshtari, F., & Omidi, A. (2021). Comparison of the effects of transcranial direct current stimulation and mindfulness-based stress reduction on mental fatigue, quality of life and aggression in mild traumatic brain injury patients: A randomized clinical trial. *Annals of General Psychiatry, 20*(1), 33.

Solomons, C. D., & Shanmugasundaram, V. (2019). A review of transcranial electrical stimulation methods in stroke rehabilitation. *Neurology India, 67*(2), 417–423.

Song, X., Zhao, X., Li, X., Liu, S., & Ming, D. (2021). Multi-channel transcranial temporally interfering stimulation (tTIS): Application to living mice brain. *Journal of Neural Engineering, 18*(3), 036003.

Song S, Zhang J, Tian Y, Wang L, and Wei P. Temporal interference stimulation regulates eye movements and neural activity in the mice superior colliculus. *43rd Annual International Conference of the IEEE Engineering in Medicine & Biology Society (EMBC)*, 2021: 6231–6234, 2021a.

Song. Typical electrode configuration analysis for temporally interfering deep brain stimulation. *9th International IEEE/EMBS* Conference *on Neural Engineering (NER)*: 770–773, 2019.

Spooner, R. K., Wiesman, A. I., & Wilson, T. W. (2022). Peripheral somatosensory entrainment modulates the cross-frequency coupling of movement-related theta-gamma oscillations. *Brain Connectivity, 12*(6), 524–537.

Storm, H. (2008). Changes in skin conductance as a tool to monitor nociceptive stimulation and pain. *Current Opinion in Anaesthesiology, 21*(6), 796–804.

Sunshine, M. D., Cassarà, A. M., Neufeld, E., Grossman, N., Mareci, T. H., Otto, K. J., Boyden, E. S., & Fuller, D. D. (2021). Restoration of breathing after opioid overdose and spinal cord injury using temporal interference stimulation. *Communications Biology, 4*(1), 107.

Syrjala E, Jiang M, Pahikkala T, Salantera S, and Liljeberg P. Skin conductance response to gradual-increasing experimental pain. In *2019 41st Annual International Conference of the IEEE Engineering in Medicine and Biology* Society *(EMBC)*: IEEE, 2019.

Tadini, L., El-Nazer, R., Brunoni, A. R., Williams, J., Carvas, M., Boggio, P., Priori, A., Pascual-Leone, A., & Fregni, F. (2011). Cognitive, mood, and electroencephalographic effects of noninvasive cortical stimulation with weak electrical currents. *The Journal of ECT, 27*(2), 134–140.

Thielscher A, Antunes A, and Saturnino GB. Field modeling for transcranial magnetic stimulation: a useful tool to understand the physiological effects of TMS? *37th annual international conference of* the *IEEE engineering in medicine and biology society (EMBC)*, 2015.

Turi, Z., Ambrus, G. G., Janacsek, K., Emmert, K., Hahn, L., Paulus, W., & Antal, A. (2013). Both the cutaneous sensation and phosphene perception are modulated in a frequency-specific manner during transcranial alternating current stimulation. *Restorative Neurology and Neuroscience, 31*(3), 275–285.

Turi, Z., Ambrus, G. G., Ho, K.-A., Sengupta, T., Paulus, W., & Antal, A. (2014). When size matters: Large electrodes induce greater stimulation-related cutaneous discomfort than smaller electrodes at equivalent current density. *Brain stimulation, 7*(3), 460–467.

Turi Z, Mittner M, Lehr A, Bürger H, Antal A, and Paulus W. 2020 θ-γ cross-frequency transcranial alternating current stimulation over the trough impairs cognitive control. *Eneuro* 7 (5)

Turner, D. A., Degan, S., Galeffi, F., Schmidt, S., & Peterchev, A. V. (2021). Rapid, Dose-dependent enhancement of cerebral blood flow by transcranial AC stimulation in mouse. *Brain Stimulation, 14*(1), 80–87.

Vanderhasselt, M.-A., de Raedt, R., Namur, V., Lotufo, P. A., Bensenor, I. M., Boggio, P. S., & Brunoni, A. R. (2015). Transcranial electric stimulation and neurocognitive training in clinically depressed patients: A pilot study of the effects on rumination.

Progress in Neuro-Psychopharmacology & Biological Psychiatry, 57, 93–99.

Verhoeven, K., & van Dijk, J. G. (2006). Decreasing pain in electrical nerve stimulation. *Clinical Neurophysiology, 117*(5), 972–978.

von Conta, J., Kasten, F. H., Schellhorn, K., Ćurčić-Blake, B., Aleman, A., & Herrmann, C. S. (2022). Benchmarking the effects of transcranial temporal interference stimulation (tTIS) in humans. *Cortex, 154*, 299–310.

Wang, Y., Shi, L., Dong, G., Zhang, Z., & Chen, R. (2020). Effects of transcranial electrical stimulation on human auditory processing and behavior-A review. *Brain Sciences, 10*(8), 531.

Wang, B., Aberra, A. S., Grill, W. M., & Peterchev, A. V. (2023). Responses of model cortical neurons to temporal interference stimulation and related transcranial alternating current stimulation modalities. *Journal of Neural Engineering, 19*(6), 66047.

Ward, A. R., & Robertson, V. J. (1998). Sensory, motor, and pain thresholds for stimulation with medium frequency alternating current. *Archives of Physical Medicine and Rehabilitation, 79*(3), 273–278.

Wiech, K. (2016). Deconstructing the sensation of pain: The influence of cognitive processes on pain perception. *Science, 354*(6312), 584–587.

Wiech, K., Ploner, M., & Tracey, I. (2008). Neurocognitive aspects of pain perception. *Trends in Cognitive Sciences, 12*(8), 306–313.

Wiesenfeld-Hallin, Z. (2005). Sex differences in pain perception. *Gender Medicine, 2*(3), 137–145.

Wischnewski, M., Schutter, D. J. L. G., & Nitsche, M. A. (2019). Effects of beta-tACS on corticospinal excitability: A meta-analysis. *Brain Stimulation, 12*(6), 1381–1389.

Yavari, F., Jamil, A., Mosayebi Samani, M., Vidor, L. P., & Nitsche, M. A. (2018). Basic and functional effects of transcranial electrical stimulation (tES)-An introduction. *Neuroscience and Biobehavioral Reviews, 85*, 81–92.

Zaehle, T., Sandmann, P., Thorne, J. D., Jäncke, L., & Herrmann, C. S. (2011). Transcranial direct current stimulation of the prefrontal cortex modulates working memory performance: Combined behavioural and electrophysiological evidence. *BMC Neuroscience, 12*(1), 2.

Zeng, F.-G., Tran, P., Richardson, M., Sun, S., & Xu, Y. (2019). Human sensation of transcranial electric stimulation. *Scientific Reports, 9*(1), 1–12.

Zhang, K., Guo, L., Zhang, J., An, G., Zhou, Y., Lin, J., Xing, J., Lu, M., & Ding, G. (2019). A safety study of 500 μA cathodal transcranial direct current stimulation in rat. *BMC Neuroscience, 20*(1), 40.

Zhang, Z., Lin, B.-S., Wu, C.-W.G., Hsieh, T.-H., Liou, J.-C., Li, Y.-T., & Peng, C.-W. (2022). Designing and pilot testing a novel transcranial temporal interference stimulation device for neuromodulation. *IEEE Transactions on Neural Systems and Rehabilitation Engineering, 30*, 1483–1493.

Zhu, Z., Xiong, Y., Chen, Y., Jiang, Y., Qian, Z., Lu, J., Liu, Y., & Zhuang, J. (2022). Temporal interference (TI) stimulation boosts functional connectivity in human motor cortex: A comparison study with transcranial direct current stimulation (tDCS). *Neural Plasticity, 2022*, 7605046.