**Article title:**

Effects of 400 Hz Transcranial Pulsed Current Stimulation on Corticospinal and Corticocortical Excitability and Hand Dexterity: A Double-Blind RCT

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Conflict of Interest: None

**Abstract:**

The effects of 400 Hz anodal and cathodal transcranial pulsed current stimulation of the primary motor cortex (400 Hz a-tPCSM1, 400 Hz c-tPCSM1) on corticospinal excitability (CSE) and corticocortical excitability (CCE) remain underexplored. This study examined the effects of 400 Hz a-tPCSM1, 400 Hz c-tPCSM1, and sham stimulation on CSE, CCE, and hand dexterity, providing insights for potential clinical applications in motor deficits.

In this double-blinded, randomized, counterbalanced crossover trial, 26 healthy young adults completed three experimental sessions: 400 Hz a-tPCSM1, 400 Hz c-tPCSM1, and sham stimulation, spaced 48 hours apart. Transcranial magnetic stimulation assessed CSE and CCE, while the Purdue Pegboard Test (PPT) evaluated hand dexterity. The results showed polarity-specific effects. A single session of 400 Hz a-tPCSM1 significantly increased CSE and improved hand dexterity, evidenced by faster PPT completion times (p < 0.05). Conversely, 400 Hz c-tPCSM1 reduced CSE but did not influence PPT performance (p > 0.05). Sham stimulation showed no significant changes. These findings suggest that 400 Hz a-tPCSM1 enhances motor excitability and dexterity, while 400 Hz c-tPCSM1 selectively reduces CSE. This study lays a foundation for exploring high-frequency tPCS in clinical motor rehabilitation.

**Key words:**

Transcranial pulsed current stimulation, Corticospinal excitability, Corticocortical excitability, Dexterity, transcranial magnetic stimulation, TMS

**Introduction**

Transcranial pulsed current stimulation (tPCS) has emerged as a promising non-invasive brain stimulation (NIBS) technique that offers an alternative to more established neuromodulatory methods, such as transcranial direct current stimulation (tDCS) and transcranial magnetic stimulation (TMS) [1-4]. Among various stimulation protocols, anodal tPCS (a-tPCS) has shown potential in modulating neuronal activity and plasticity, affecting both cognitive and motor functions [3, 4]. Despite the progress made in the field, most research has primarily concentrated on the effects of low-frequency stimulation protocols, with limited exploration of high-frequency tPCS protocols [5-7].

Expanding the stimulation frequencies above 100 Hz is expected to enhance the precision of plasticity modulation and offers potential for therapeutic interventions targeting neurological conditions associated with pathological oscillations and motor impairments.

Neuronal oscillations at specific frequencies have been linked to various cognitive and sensorimotor functions, such as attention and memory [8]. One notable example is the "ripples" oscillation, typically occurring within the 80–200 Hz frequency range, predominantly in the hippocampus and para hippocampal regions, and implicated in both normal behaviors and pathological conditions like epilepsy [9, 10]. A subset of these, termed "fast ripples," occurs between 250–500 Hz and is proposed as a biomarker for epileptic tissue [11]. These high-frequency oscillations have also been recorded in healthy neocortical areas, but they are absent in the hippocampus and are thought to reflect more specific cortical activity [12, 13].

Human non-invasive brain stimulation studies have largely focused on traditional EEG frequency bands [14, 15], as well as on higher gamma frequencies involved in cortical binding [16]. However, expanding the frequency range to over 100 Hz may enable more targeted modulation of cortical excitability, offering valuable therapeutic potential for neurological diseases with abnormal oscillatory activity and motor dysfunction [17, 18]. In particular, frequencies in the ripple range (120–130 Hz) have already been successfully utilized in deep brain stimulation for Parkinson's disease, targeting the afferent pathways to the subthalamic nucleus [17, 18].

The primary motor cortex (M1) is a critical area involved in motor control and has long been a focus of NIBS research [2]. High-frequency stimulation at 400 Hz, whether anodal or cathodal, is proposed to modulate cortical excitability and may improve motor functions, particularly for tasks requiring fine motor control, such as hand dexterity. Fine motor skills rely heavily on neural coordination, with synchronized neuronal firing across cortical and subcortical regions. Oscillations at fast ripple range play an essential role in motor coordination and precision, and abnormal high frequency patterns are linked to motor disorders like Parkinson's disease [19]. Therefore, investigating the effects of 400 Hz tPCS over M1 offers potential insights into enhancing motor function, particularly in tasks requiring precise movements.

The rationale for using 400 Hz anodal and cathodal tPCS over M1 (400 Hz a-tPCSM1 and 400 Hz c-tPCSM1) could be based on its potential to preferentially target fast-spiking interneurons that regulate cortical excitability and plasticity, directly influencing motor control pathways [20]. By modulating these fast-spiking circuits, 400 Hz a-tPCSM1 may enhance the precision of motor commands, leading to improved motor performance. While high frequency tPCS shows promise, empirical evidence on its effects in both healthy individuals and clinical populations remains sparse [21]. Most research has concentrated on therapeutic applications involving low-frequency stimulation, leaving a significant gap in our understanding of the specific effects of 400 Hz a-tPCS on the neurophysiological mechanisms underpinning motor function.

This study aims to systematically explore the effects of 400 Hz a-tPCS and cathodal tPCS (c-tPCS) on corticospinal excitability (CSE), intracortical facilitation (ICF), short-interval intracortical inhibition (SICI), long-interval intracortical inhibition (LICI), and motor performance, specifically hand dexterity, as assessed by the Purdue Pegboard Test (PPT) in healthy young adults. Additionally, the safety and tolerability of high-frequency tPCS will be evaluated. By elucidating the neurophysiological effects of 400 Hz tPCS and its impact on motor function, this study will contribute to the development of novel NIBS protocols and expand treatment options for neurological and psychiatric conditions.

**Materials and Methods**

1. **Study Design and Participants**

This study is a double-blinded, randomized, counterbalanced crossover trial designed to evaluate the effects of dual 400 Hz a-tPCS of M1 and cerebellum on the CSE, ICF, SICI and LICI and hand dexterity in healthy young adults (Figure 1). A total of 26 right-handed healthy young adults from Monash University, (Melbourne, Australia) students randomly participated in three experimental sessions: (1) 400 Hz a-tPCSM1/c-tPCSCB, (2) 400 Hz c-tPCSM1/a-tPCSCB and (3) Sham 400 Hz a- and c-tPCS M1 and CB. Following each experimental condition, participants underwent a washout period of at least 48 hours to mitigate carry-over effects. Participants were randomized using a computer-generated sequence to receive both the active and sham interventions in a counterbalanced order.

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The study procedures were reviewed and approved by the Human Ethics Committee at Monash University (approval number: 32335, May 2023) and adhered strictly to ethical guidelines, including the 1964 Helsinki Declaration and its subsequent amendments. Participants received comprehensive information about the study’s purpose, procedures, potential risks, and their rights, including the right to withdraw at any time without consequences. Written informed consent was obtained from all participants before their involvement in the study.

**Inclusion/Exclusion criteria**

Participants aged 18 to 40 years with intact cognitive abilities, no history of neurological or psychiatric disorders, no contraindications to non-invasive brain stimulation (NIBS) or transcranial magnetic stimulation (TMS), and not using neuroactive medications were included in the study. Exclusion criteria included metallic implants in the head, indwelling body stimulators, a history of seizures, or current pregnancy.

**Interventions**

All experimental sessions are carried out using the AscenZ IV Multi-channel Pulsed Current Stimulator (AscenZion Neuromodulation Co Pte Ltd, Singapore) (Figure 2A).

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The stimulator is approved as a medical device by the Health Sciences Authority of Singapore. A 4-cm diameter circular silver-fiber cotton electrode (Figure 2B) (AscenZion Neuromodulation Co Pte Ltd, Singapore) was used and positioned over the M1 area corresponding to the right first dorsal interosseous (FDI) muscle (C3), as determined by the 10-20 international EEG system (Figure 2B). The return electrode was placed on the contralateral shoulder. During active stimulation, participants received 400 Hz a-tPCSM1 and 400 Hz c-tPCSM1 at an intensity of 0.6 mA for 20 minutes [21].

**Sham intervention**

The procedure began with a brief 30-second 400 Hz anodal or cathodal tPCSM1 stimulation, followed by a 19-minute control period without stimulation, and concluded with another 30-second 400 Hz anodal or cathodal tPCSM1 stimulation (Figure 2C). The AscenZ IV stimulator employed an automated, structured approach that mimicked the timing of real tDCS while minimizing actual stimulation to serve as a sham control.

**Outcome Measures**

**CSE:** Motor-evoked potentials (MEPs) were generated using transcranial magnetic stimulation (TMS) and recorded from the FDI muscle through electromyography (EMG) at both baseline and immediately following the intervention. The peak-to-peak amplitude of the MEPs, which reflects CSE, was used as the primary outcome measure.

**CCE:** Alterations in CCE were evaluated using paired-pulse TMS, specifically assessing ICF, SICI, and LICI at the same intervals as CSE measurements.

**Hand Dexterity:** The primary outcome measure of hand dexterity was the time taken to complete each trial of the PPT, which reflects the participants' efficiency in manipulating and placing pegs.

**TMS assessment of CSE**

Participants were seated comfortably in a treatment chair (MagVenture, Denmark) with their dominant hand rested on a pillow in a prone position to ensure muscle relaxation. After skin preparation to reduce impedance, EMG electrodes were placed on the FDI muscle per SENIAM (Surface ElectroMyoGraphy for the Non-Invasive Assessment of Muscles) guidelines, with reference electrodes on a nearby bony prominence.

Single-pulse TMS induced MEPs were recorded from the dominant FDI muscle at rest using bipolar Ag/AgCl surface electrodes, spaced 2 cm apart. A ground electrode was placed over the ulnar styloid process. EMG signals were filtered (10-500 Hz), amplified (x 1000), and sampled at 1000 Hz, with data recorded via LabChart™ software using a Powerlab analog-digital interface (Powerlab, ADInstruments, Australia).

The TMS coil was positioned over the motor cortex (C3) to identify the optimal stimulation site (hotspot) for the FDI muscle. Resting motor threshold (RMT) was determined as the minimum intensity needed to produce a detectable MEP in at least 50% of trials. Single-pulse TMS was then applied at 120% of RMT to record 25 MEPs, with average peak-to-peak amplitude serving as the CSE index. Safety guidelines were observed throughout.

**TMS assessment of CCE (SICI, LICI and ICF)**

Motor evoked potentials (MEPs) elicited by paired-pulse TMS were recorded from the dominant FDI muscle at rest. A conditioning stimulus (CS) was delivered at 80% of the RMT, and a test stimulus (TS) was adjusted to produce an MEP of approximately 1 mV (test MEP). For SICI, the interval between CS and TS was set to 3 ms, while for ICF, the interval was set to 10 ms. The ratio of these conditioned MEPs to the test MEP reflects the level of SICI and ICF. For SICI, a reduced conditioned MEP amplitude indicated inhibition, while for ICF, an increased conditioned MEP amplitude indicated facilitation. LICI was assessed by delivering two TMS pulses with a 150 ms interval, where a reduction in the conditioned MEP amplitude signalled LICI.

**Assessment of Hand dexterity**

The PPT was used to assess participants' fine motor skills before and after interventions. This test involves placing small pegs into holes on a pegboard, emphasizing speed and precision. Three trials are conducted, each focusing on peg placement with the participant’s dominant hand. Completion times are recorded and averaged, providing a measure of manual dexterity to analyse motor skill performance across study time points.

**Procedure**

The three interventions were conducted in randomised order. Data collection order was randomised across all three testing sessions. Participants refrained from drinking alcohol, coffee or energy drinks at least 12-hours prior to participating to avoid effects of ethanol [22, 23] and caffeine [24, 25] on CSE and CCE. A minimum 48-hour washing-out period eliminated the risk of carry-over effects [26]. Each session was conducted at a similar time-of-day to minimise cortisol diurnal effects on CSE and CCE [27, 28].

Once participants were seated and the RMT and the CI and TI were established, baseline outcome measures were recorded. Following this, one of the three 400 Hz tPCSM1 interventions was administered randomly. Then again outcome measures were recorded immediately post-intervention. Single-pulse MEPs were recorded at the original baseline TI. The RMT and TI required to elicit an MEP amplitude of approximately 1 mV were then re-determined. These new values were used to record paired-pulse MEPs for calculating SICI, LICI, and ICF.

**Blinding**

In this crossover study, a double-blind design was used to assess the effects of 400 Hz anodal, cathodal, and sham tPCS over M1 in three separate sessions. Participants were randomly assigned to receive one of the three treatments in each session, with conditions counterbalanced across subjects. The blinding ensured that participants were unaware of which condition they received at any point. Additionally, the researcher responsible for statistical analysis was blinded to the treatment assignments to prevent any bias in the evaluation of outcomes.

**Statistical analysis**

Data analysis for this study was conducted using GraphPad Prism 10. Before the primary analyses, a one-way ANOVA compared baseline levels of CSE, ICF, SICI, LICI, and pegboard completion time across the three conditions: 400 Hz a-tPCSM1, 400 Hz c-tPCSM1 and sham stimulation. This check ensured no baseline differences among groups and no carryover effects between sessions. If significant differences (p < 0.05) were found, post-hoc Tukey's tests were conducted to identify specific group differences.

Then repeated-measures analysis of variance (ANOVA) was used to assess the effects of a single session of 400 Hz a-tPCSM1 and 400 Hz c-tPCSM1 on different outcome measures. Separate 2x2 ANOVAs (2 stimulation conditions x 2 time points) examined the effects of anodal or cathodal tPCS on CSE, ICF, SICI, LICI, and PPT. Post-hoc pairwise comparisons were performed to further examine the differences among the multiple comparisons, utilizing Bonferroni correction for adjustment. Statistical significance was set at p < 0.05 for all analyses. All results were reported with corresponding p-values, F-statistics, and effect sizes to ensure clarity in interpretation.

**Results**

**Participants**

The study involved 26 healthy participants (16 female, 10 male) with a mean age of 26.7 years (SD = 4.7, range = 20-36), recruited from university students and community volunteers.

In this crossover design, participants underwent three stimulation conditions, starting with one condition randomly assigned, with baseline comparisons confirming balanced groups.

**Baseline Data**

Baseline measurements for CSE, SICI, LICI, ICF, and PPT completion time were recorded across the three experimental conditions. A one-way repeated-measures ANOVA was conducted to determine any significant differences in baseline values among the conditions. The results revealed no significant differences in baseline measurements for any of the neurophysiological measures (Table 1):

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These findings indicate that baseline neurophysiological measures were consistent across all conditions, confirming no carryover effects between sessions and ensuring that any observed effects in later phases are not due to baseline differences.

**The effects of 400 Hz a-tPCSM1 on CSE, ICF, SICI, and LICI**

Figure 3A illustrates the effects of 400 a-tPCS on CSE compared to sham stimulation. The results show that a-tPCS of M1 significantly increases CSE immediately after stimulation, while sham stimulation did not lead to any notable changes. This indicates that active stimulation produces significant effects on CSE, unlike the sham condition.

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Statistical analysis using a two-way repeated measures ANOVA (2 stimulation conditions × 2 time points) for each outcome measure revealed a significant interaction effect (Stimulation type × Time) for all measures except ICF (CSE: F(1, 44) = 11.33, p = 0.0016; SICI: F(1, 44) = 4.59, p = 0.037; LICI: F(1, 44) = 9.15, p = 0.004; ICF: F(1, 44) = 3.48, p < 0.068). Post hoc pairwise comparisons were performed using Bonferroni correction.

*CSE:* Anodal tPCS applied to the M1 significantly increased CSE compared to sham stimulation (p < 0.001), indicating enhanced corticospinal output following stimulation (Figure 3A). Sham stimulation had no significant effect on CSE (p = 0.87).

*ICF:* ICF was significantly increased following a-tPCS compared to sham stimulation (p < 0.001), demonstrating enhanced excitatory synaptic activity within the motor cortex (Figure 3C). Sham stimulation did not affect ICF (p = 0.92).

*SICI:* SICI was significantly reduced following a-tPCS relative to sham (p = 0.004) (Figure 3B), indicating decreased inhibitory GABAergic activity. No significant changes in SICI were observed in the sham condition (p = 0.76).

*LICI:* Anodal tPCS also significantly reduced LICI compared to sham (p = 0.008), implying a decrease in intracortical inhibition (Figure 3C). The sham condition did not result in significant alterations in LICI (p = 0.82).

**The effects of 400 Hz c-tPCSM1 on CSE, ICF, SICI, and LICI**

The graph in Figure 4A shows the effects of 400 Hz c-tPCSM1 on CSE compared to sham stimulation. The statistical analysis revealed a significant main effect of stimulation type was found for CSE and LICI, while no significant effects were observed for SICI and ICF (CSE: F(1, 44) = 4.45, p = 0.04; LICI: F(1, 44) = 8.90, p = 0.004; SICI: F(1, 44) = 2.93, p = 0.093; ICF: F(1, 44) = 0.88, p = 0.351).

<<<Please insert figure 4 here>>>

*CSE:* CSE significantly decreased following c-tPCS compared to sham stimulation (p = 0.001). This indicates reduced corticospinal output after cathodal stimulation. Sham stimulation did not result in any significant changes in CSE (p = 0.84).

*ICF:* There were no significant changes in ICF following c-tPCS compared to sham stimulation (p > 0.05). Sham stimulation did not influence ICF (p > 0.05), indicating that excitatory intracortical circuits remained unaffected by both conditions.

These results demonstrate that 400 Hz c-tPCS significantly decreases CSE and increases LICI, while no significant effects were observed for SICI or ICF. Sham stimulation produced no significant changes across all measures, as illustrated in Figure 4.

*SICI:* Although there was an increase in SICI following c-tPCS, this change was not statistically significant (p = 0.14). Sham stimulation had no significant effect on SICI (p = 0.77), indicating that neither condition robustly influenced this measure of GABAergic inhibition.

*LICI*: A significant increase in LICI was observed following c-tPCS compared to sham (p = 0.004), suggesting enhanced inhibitory control within the motor cortex. Sham stimulation produced no significant effect on LICI (p = 0.89).

**The Effects of 400 Hz anodal and cathodal tPCSM1 on PPT completion time**

Figure 5 illustrates the effects of 400 Hz a-tPCS and c-tPCS on PPT completion time compared to sham stimulation. Statistical analysis revealed a significant main effect for a-tPCS only (F (2, 72) = 5.41, p = 0.006), while cathodal (p > 0.05) and sham stimulation (p > 0.05) showed no significant effects.

Anodal tPCS significantly decreased completion time, indicating improved fine motor skills and dexterity, thus enhancing motor performance on the PPT.

In contrast, cathodal tPCS did not significantly change completion time compared to sham (p > 0.05), indicating no improvement. Sham stimulation also showed no significant changes, serving as a control.

**Side and Adverse Effects**

Side effects of anodal, cathodal, and sham 400 Hz tPCSM1 were recorded during the 20-minute application of 400 Hz tPCS and up to 30 minutes following its completion (Figure 5).

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Participants in both anodal and cathodal tPCS conditions reported mild side effects, including tingling, itching, burning sensations, and headaches, each rated below 2 on a 10-point Visual Analog Scale (VAS) under the anode, regardless of the stimulation site. These sensations were less pronounced under the cathode across both active and return electrodes. During sham stimulation, side effect severity was further reduced, with participants reporting near-zero levels of side effects at both active and return electrode sites.

**Discussion**

This study explored the effects of 400 Hz tPCSM1 on key neurophysiological measures, including CSE, SICI, LICI, and ICF, as well as hand dexterity assessed by the PPT. The comparison between anodal, cathodal, and sham stimulation provides novel insights into the differential effects of 400 Hz tPCS on motor cortical function and motor performance.

**The effects of 400 Hz a-tPCSM1**

*CSE:* The results showed a significant increase in CSE after 400 Hz a-tPCSM1, consistent with previous findings that anodal stimulation enhances pyramidal cell depolarization and increases MEP amplitudes [2]. This indicates that 400 Hz a-tPCSM1 may boost excitatory drive in the corticospinal tract, benefiting motor rehabilitation.

*ICF:* Anodal tPCS significantly increased ICF, indicating enhanced intracortical facilitation likely mediated by NMDA receptor activity, which supports its overall excitatory effects on motor cortical function [29]. As expected, sham stimulation did not lead to changes in ICF, reinforcing previous findings that sham protocols do not influence excitatory processes in the cortex [30]. These results emphasize that meaningful changes in CSE depend on active stimulation, underscoring the specificity of our experimental design and the role of active stimulation in eliciting neurophysiological responses related to motor function [31].

*SICI*: Our findings indicate that anodal tPCS significantly reduced SICI, reflecting decreased intracortical inhibition likely due to modulation of GABAergic activity (GABA A), which underlies SICI [32]. This reduction in SICI suggests enhanced excitatory synaptic activity, contributing to the overall increase in CSE following anodal stimulation. Sham stimulation did not produce significant changes in SICI or corticospinal excitability, confirming that this condition, which simulates stimulation without active electrical currents, did not meaningfully modulate inhibitory intracortical networks [30].

*LICI*: Our findings indicate that a-tPCS significantly reduces LICI, suggesting decreased intracortical inhibition, likely due to modulation of GABA\_B receptors, which are crucial for LICI mechanisms [32-34]. The reduction in LICI may reflect enhanced excitatory synaptic activity, contributing to the overall increase in CSE following a-tPCS. This relationship highlights the complex dynamics of cortical excitability and potential therapeutic pathways for motor rehabilitation [35, 36].

**The mechanisms driving the effects of 400 Hz a-tPCSM1 on CSE and CCE**

Fast ripple frequencies (200–600 Hz) in the cortex are associated with excitatory effects primarily through synchronized neuronal activity, driven by synaptic mechanisms and intrinsic bursting. Neurons, particularly pyramidal cells, involved in fast ripple activity often exhibit intrinsic bursting properties, which amplify excitatory signaling. This is facilitated by the activation of glutamatergic synapses, especially AMPA receptors, enhancing neuronal communication and network synchronization [37, 38]. Furthermore, these intrinsic bursts recruit neighboring neurons, promoting synchronized firing and contributing to the hallmark oscillatory patterns of fast ripples [38, 39].

Additionally, high-frequency transcranial peripheral current stimulation (tPCS) has been shown to influence fast-spiking interneurons in the motor cortex, thereby fine-tuning motor outputs. This enhances motor performance and precision during tasks by modulating motor pathways involving parvalbumin-positive (PV+) interneurons, which regulate pyramidal neuron activity. These interneurons play a critical role in corticospinal excitability and motor control [18, 40]. Targeting these mechanisms, such as with 400 Hz a-tPCSM1, offers a promising neuromodulatory approach to improve motor function.

**The effects of 400 Hz c-tPCSM1**

*CSE:* The results showed a significant decrease in CSE after 400 Hz a-tPCSM1, reflecting its inhibitory effects. This finding is in line with the finding of a-tPCSM1 at lower-frequencies [3]. Sham stimulation showed no significant changes in excitability, reinforcing the specificity of active stimulation effects.

*ICF:* 400 Hz c-tPCSM1 did not induced significant changes in ICF, suggesting it primarily influences inhibitory rather than excitatory intracortical circuits. This aligns with research indicating that cathodal stimulation suppresses cortical excitability, enhancing inhibitory mechanisms without markedly affecting facilitatory pathways [2, 32]. The lack of significant alteration in ICF further supports the idea that 400 Hz c-tPCSM1 fine-tunes cortical inhibition without enhancing excitatory synaptic activity [35].

*SICI:* 400 Hz c-tPCSM1 resulted in a non-significant increase in SICI, reflecting enhanced inhibition due to the hyperpolarizing effects of cathodal stimulation. This increased intracortical inhibition may account for the slight reduction in CSE observed in the cathodal group.

*LICI:* The application of 400 Hz c-tPCSM1 resulted in a significant increase in long-interval intracortical inhibition (LICI). This finding suggests an enhancement of inhibitory processes mediated by GABA\_B receptors within the motor cortex.

In alignment with the findings on SICI, the sham stimulation condition did not induce significant changes in LICI or corticospinal excitability. This confirms that the sham protocol, designed to replicate the sensory experience of active stimulation without delivering actual electrical currents, does not alter inhibitory intracortical networks. Such results validate the effectiveness of the sham setup as a control, ensuring that observed effects in active conditions are attributable to the delivered stimulation rather than placebo or nonspecific factors. [30]. These results underscore the importance of active stimulation in eliciting neurophysiological changes and reaffirm the validity of our experimental controls.

**The mechanisms driving the effects of 400 Hz c-tPCSM1 on CSE and CCE**

400 Hz c-tPCSM1 appears to modulate CSE through mechanisms involving enhanced inhibitory processes mediated by GABAergic pathways. Specifically, this high-frequency stimulation has been linked to an increase in LICI, which reflects GABA\_B-mediated inhibitory activity in the motor cortex [41, 42]. 400 Hz c-tPCSM1 may preferentially target fast-spiking interneurons, such as parvalbumin-positive (PV+) GABAergic interneurons, which play a crucial role in maintaining inhibitory control and regulating cortical excitability [20, 40]. By enhancing the activity of these interneurons, cathodal stimulation strengthens the balance between excitation and inhibition, which results in a reduction of CSE [43]. Moreover, the modulation of synaptic plasticity mechanisms, such as long-term depression (LTD), is thought to contribute to the inhibitory effects observed during 400 Hz c-tPCSM1, suppressing excitatory drive within corticospinal pathways [43]. These findings suggest that high-frequency cathodal tPCS could be a promising tool for downregulating hyperactive motor cortex states, which are often seen in conditions such as spasticity or focal dystonia [44].

**Hand Dexterity**

The effects of 400 Hz tPCS on hand dexterity, assessed by the PPT, revealed important behavioral insights. Anodal tPCS significantly improved performance, suggesting that increased CSE and reduced SICI enhance motor function. This aligns with prior research indicating that boosting cortical excitability can improve fine motor skills [45], which is especially relevant in rehabilitation contexts focused on motor outcomes.

In contrast, cathodal tPCS did not yield significant improvements in PPT performance, likely due to increased inhibition (SICI and LICI). Similarly, sham stimulation failed to enhance performance, underscoring the unique effects of anodal stimulation on motor skills. This highlights the necessity of active stimulation for facilitating motor skill improvement.

**Limitations and suggestions for future research**

This study offers valuable insights into the effects of 400 Hz tPCS on corticospinal excitability, intracortical inhibition, and motor performance, but several limitations must be acknowledged. First, the study did not examine gender effects. Research indicates that sex hormones can influence cortical excitability and neuroplasticity, potentially leading to different responses to tPCS between men and women [46]. Future studies should recruit a gender-balanced sample to explore possible sex-based differences in the impact of 400 Hz tPCS on motor function. Age-related differences are also a limitation, as participants were relatively young adults, which may limit the findings' applicability to older populations. Since CSE and the interaction of inhibitory or facilitatory mechanisms can change with age [47], it is crucial to assess whether similar effects occur in older adults, who may show different motor performance outcomes. Future studies should include participants from a broader age range to examine how aging influences responses to tPCS.

Moreover, this study only assessed the immediate effects of tPCS. While changes in CSE and motor function were noted, it remains uncertain whether these effects are sustained over time. Long-term follow-up assessments are necessary to understand the duration of 400 Hz tPCS effects on motor function and neuroplasticity. Finally, the research focused solely on motor cortical measures and did not investigate potential effects of 400 Hz tPCS on cognitive or sensory domains. Future research should explore whether this stimulation frequency impacts broader cortical networks and influences cognitive functions or sensory processing.

In summary, while this study presents promising initial results, the limitations regarding gender, age, and short-term assessments highlight the need for further research to enhance findings and explore broader applications of 400 Hz tPCS.

**Clinical Implications**

The differential effects of 400 Hz tPCS on neurophysiological and behavioral outcomes suggest potential therapeutic applications. Anodal tPCS may be beneficial in motor rehabilitation, where enhancing cortical excitability and reducing intracortical inhibition could improve fine motor skills in populations with motor deficits, such as those recovering from stroke or neurological conditions. Conversely, the inhibitory effects of cathodal tPCS may be applicable in conditions where reducing cortical excitability is desirable, such as chronic pain or epilepsy, where cortical hyperexcitability is a concern.

Further research is needed to explore the long-term effects of 400 Hz tPCS and its potential in various clinical populations. Improvements in hand dexterity following anodal stimulation indicate this modality's potential to enhance functional outcomes in motor rehabilitation.

**Conclusions**

This study provides strong evidence for the differential effects of 400 Hz tPCS on CSE and intracortical inhibition across anodal, cathodal, and sham stimulation. A-tPCS significantly increased CSE and reduced SICI, leading to improved hand dexterity. This suggests that anodal stimulation can enhance motor function, indicating its potential for motor rehabilitation.

In contrast, cathodal tPCS slightly decreased corticospinal excitability, increased SICI and LICI, highlighting heightened inhibitory processes in the motor cortex. The lack of improvement in motor dexterity with cathodal stimulation suggests it may be more suitable for conditions like chronic pain or epilepsy. The sham group showed no significant changes in measured parameters, confirming the specificity of the active stimulation effects.

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**Author Contributions**

Conceived and designed study: SJ, MZ, MM and RL; Carried out data collection: MM and RL; Conducted the analysis: SJ; Interpreted the findings: SJ, MZ; Wrote the Manuscript: SJ; Writing and editing of drafts: SJ, MZ, MM and RL.

**Data Availability Statement**  
The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request. All relevant data supporting the findings of this study are included in this published article. Any additional datasets that are not publicly available are restricted due to confidentiality and ethical restrictions but can be provided upon reasonable request subject to the approval of Monash Human Research committee, if applicable.

**Conflict of Interest Statement**

The authors declare no commercial or financial relationships that could be construed as a potential conflict of interest.

**Funding**

This work was supported by AscenZion Neuromodulation Co. Pte. Ltd, Singapore, which had no influence on the study design, data collection, analysis, or publication process.

**The use of generative AI and AI-assisted technologies**

During the preparation of this work the author(s) used ChatGPT in the writing process to improve the readability and language of the manuscript. After using this tool, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the published article.

**Figure legends**

**Figure 1.** Study setup. Each participant attends all three experimental conditions with 48-hour washing out period. CSE, SICI, LICI and ICF and hand dexterity will be assessed before and immediately after the three experimental conditions.

**Figure 2:** (A) Parameters of 400 Hz tPCS, including pulse duration, inter-pulse interval, net direct current component, and duty cycle. (B) AscenZ-IV tPCS stimulator, circular silver fibre cotton electrode, and hydrogel electrode pad. (C) Setup for 400 Hz sham anodal/cathodal tPCSM1.

**Figure 3:** The effects of 400 Hz a-tPCS on CSE, ICF, SICI and LICI compared to sham stimulation. The dotted line at the ratio of 100% represents a baseline or control level of MEP amplitude. This line serves as a reference point, indicating the level of MEP response without any inhibitory or facilitatory conditioning stimulus applied. \*p < 0.05; \*\*p < 0.01. Con.: Conditioned, MEP: Motor Evoked Potentials, Test MEP: CSE: Corticospinal Excitability, ICF: Intracortical Facilitation, SICI: Short Interval Intracortical Inhibition, LICI: Long Interval Intracortical Inhibition.

**Figure 4**: the effects of 400 Hz c-tPCS on CSE, ICF, SICI and LICI compared to sham stimulation. The dotted line at the ratio of 100% represents a baseline or control level of motor evoked potential (MEP) amplitude. This line serves as a reference point, indicating the level of MEP response without any inhibitory or facilitatory conditioning stimulus applied. \*p < 0.05; \*\*p < 0.01.

**Figure 5**: The side and adverse effects of anodal (A), cathodal (C), and sham 400 Hz tPCSM1 (E) under the active electrodes. It also presents the effects under the return electrodes (B, D, and F).

**Tables**

Table 1: One-Way Repeated-Measures ANOVA for Baseline Values

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| |  | | --- | | **Neurophysiological Measures** | | |  | | --- | | **F-Value**  **(df = 1, 2)** | | **p-Value** |
| |  | | --- | | CSE | | |  | | --- | | 0.39 | | |  | | --- | | 0.677 | |
| |  | | --- | | ICF | | |  | | --- | | 0.84 | | |  | | --- | | 0.435 | |
| |  | | --- | | SICI | | |  | | --- | | 0.15 | | |  | | --- | | 0.858 | |
| |  | | --- | | LICI | | |  | | --- | | 0.90 | | |  | | --- | | 0.410 | |
| |  | | --- | | PPT Completion Time | | |  | | --- | | 0.34 | | |  | | --- | | 0.710 | |

**Figure 1:**

A screenshot of a test

AI-generated content may be incorrect.

**Figure 2:**

**A screenshot of a device

AI-generated content may be incorrect.**

**Figure 3**

**A group of graphs showing different types of numbers

AI-generated content may be incorrect.**

**Figure 4**

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**Figure 5**

**A graph of electrolysis

AI-generated content may be incorrect.**