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Title page

Title: **The effects of a single session cathodal transcranial pulsed current stimulation on corticospinal excitability: a randomised sham controlled double-blinded study**

Abbreviated title: Effects of c-tPCS on corticospinal excitability

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Abstract

Transcranial pulsed current stimulation (tPCS) of the human motor cortex has received much attention in recent years. Although the effect of anodal tPCS with different frequencies has been investigated, the effect of cathodal tPCS (c-tPCS) has not been explored yet. Therefore, the aim of the present study was to investigate the effect of c-tPCS at 4 and 75 Hz frequencies on corticospinal excitability (CSE) and motor performance. In a randomized sham-controlled cross over design, fifteen healthy participants attended three experimental sessions and received either c-tPCS at 75 Hz, 4 Hz or sham with 1.5 mA for 15 min. Transcranial magnetic stimulation and grooved pegboard test were performed before, immediately after and 30 min after the completion of stimulation at rest. The findings indicate that c-tPCS at both 4 Hz and 75 Hz significantly increased CSE compared to sham. Both c-tPCS at 75 Hz and 4 Hz showed a significant increase in intracortical facilitation compared to sham, whereas the effect on short-interval intracortical inhibition was not significant. The c-tPCS at 4 Hz but not 75 Hz induced modulation of intracortical facilitation correlated with the CSE. Motor performance did not show any significant changes. These results suggest that, compared with sham stimulation, c-tPCS at both 4 Hz and 75 Hz induces an increase in CSE.

Key words: transcranial pulsed current stimulation; tPCS; transcranial magnetic stimulation; TMS; corticospinal excitability; motor performance

Abbreviations

CMS	common mode sense
CSE	corticospinal excitability
DRL	driven right leg
EEG	electroencephalography
FDI	first dorsal interosseous
GABA	gamma-aminobutyric acid
GPT	grooved pegboard test
ICF	Intracortical facilitation
LTD	long-term depression
LTP	long-term potentiation
M1	primary motor cortex
MEPs	motor evoked potentials
NMDA	N-methyl-D-aspartate
rTMS	repetitive transcranial magnetic stimulation
SICI	short-interval intracortical inhibition
STDP	spike timing dependent activity
tACS	transcranial alternating current stimulation
TMS	transcranial magnetic stimulation
tPCS	transcranial pulsed current stimulation

1 INTRODUCTION

Over the last two decades, there has been an increasing interest into investigation of the brain rhythms to modulate brain function in humans. Evidence of such activity is widely reported in the electroencephalography (EEG) frequency bands of theta (4-8 Hz) and gamma (30-100

Hz). Specifically, the low-frequency theta band is primarily categorized as non-motor rhythms. The activity of theta band has been mainly observed during attentional and cognitive tasks, during rapid eye movement (REM) sleep, and wakefulness (Klimesch, 1996; Klimesch, 1999; Kahana, 2006; Klimesch et al., 2008). In contrast, high-frequency gamma band has been identified as pro-kinetic or motor rhythm where the movements are facilitated (Brown, 2003; Joundi et al., 2012; Santarnecchi et al., 2017). Gamma band has been found to increase prior to and during motor performances and influence the reaction time (Hamada et al., 1999; Shibata et al., 1999; Brown, 2003; Muthukumaraswamy, 2011; Joundi et al., 2012; Santarnecchi et al., 2017).

The non-invasive brain stimulation techniques with rhythmic stimulation protocols provide the opportunity to modulate brain oscillations in a frequency-specific manner. Importantly, transcranial pulsed current stimulation (tPCS) has identified to have such capacity to modulate brain oscillations via entrainment (Castillo Saavedra et al., 2014; Vasquez et al., 2016). It involves the application of monophasic or biphasic pulses via electrodes placed over the target brain area (Jaberzadeh et al., 2014; Jaberzadeh et al., 2015). A previous study using monophasic anodal tPCS (a-tPCS) at theta frequency (5.7 Hz) over the primary motor cortex (M1), showed no after-effect on corticospinal excitability (CSE) (Jaberzadeh et al., 2015). Despite this, there has been a lack of literature addressing the effect of monophasic tPCS at theta frequency on CSE. However, studies have investigated the effect of transcranial alternating current stimulation (tACS) on brain oscillations (Antal et al., 2008; Feurra et al., 2013; Guerra et al., 2016). It should be noted that tACS and tPCS shares some similar characteristics (i.e., both use frequency-based pulses) where tACS literature may have implications for the investigation of tPCS. In the motor domain, a study by Feurra et al. showed no effect on CSE during tACS at 5 Hz compared to no stimulation (Feurra et al., 2013). In addition, another study using tACS at 7 Hz also showed no effect on CSE (Guerra et al., 2016). Overall, the evidence is in line with that low-frequency stimulation at theta band produces no change in CSE.

In contrast to theta frequency, the literature indicates that the higher gamma (60-100 Hz) frequency band is closely related to the excitatory activity of interneurons in M1 (Buzsaki, 1996; Wang and Buzsaki, 1996; Buzsaki, 1998). Emerging evidence also suggests that there is a link between oscillatory activity in the gamma band and the balance of excitation and inhibition within reciprocally connected networks of inhibitory GABA-ergic interneurons and excitatory glutamatergic pyramidal cells within M1 that determines the level of CSE (Atallah

and Scanziani, 2009; Gaetz et al., 2013). Several studies on tACS at gamma frequency found no after-effect on CSE (Antal et al., 2008; Nowak et al., 2017; Guerra et al., 2018; Sugata et al., 2018; Guerra et al., 2019), but reduced inhibition only during the stimulation (Nowak et al., 2017; Guerra et al., 2018; Guerra et al., 2019). Moreover, movement-related changes were also observed with tACS at gamma oscillation (Joundi et al., 2012; Moisa et al., 2016; Guerra et al., 2018; Heinrichs-Graham et al., 2018; Bologna et al., 2019). For example, it has been shown that application of tACS at 70 Hz improves hand movement velocity and acceleration during a visually guided motor task (Moisa et al., 2016). In general, these observations support the pro-kinetic nature of the gamma oscillation.

The neurophysiological effects induced by a-tPCS have been studied extensively in the motor domain (Alon et al., 2012; Jaberzadeh et al., 2014; Sours et al., 2014; Jaberzadeh et al., 2015; Luu et al., 2016). Moreover, the effect of a-tPCS on clinical symptoms of Parkinson's disease have been evaluated (Alon et al., 2012). However, the potential impact of the novel technique of cathodal tPCS (c-tPCS) remains largely unknown. C-tPCS delivers negative pulses to induce negative net direct current component (NDCC) that may lead to decreased excitability on the target brain area via polarity-dependent manner (Figure 1). In addition, it induces a frequency-dependent effect that relies on the pulsatile nature (on/off) of the current. A recent study demonstrates that c-tPCS modulates intrinsic brain oscillations, inducing frequency-specific changes in CSE (Luu et al., 2016). For example, Luu et al., (2016) showed that high-definition tPCS (both anodal and cathodal) at 0.5 Hz and 1.16 mA reduces the CSE. They have concluded that tPCS at low frequency induces long-term depression (LTD) and reduce CSE on the M1 area, regardless of the direction of the current (Luu et al., 2016). This finding highlights the importance of the frequency of tPCS on M1 excitability.

"Please insert Figure 1 here"

To date, no studies have focused on comparing the effect of c-tPCS at non-motor low-frequency theta band vs. motor-related high-frequency gamma band on M1. Indeed, given a large number of studies highlighting the importance of oscillatory changes within theta and gamma bands, it remains to be explored the effect of c-tPCS at these frequencies on CSE. It also remains undetermined whether such neurophysiological changes would affect motor performances. Thus, a clear understanding of how c-tPCS affects brain function is crucial for developing it as an effective therapeutic protocol in future. The aim of the present study was to investigate whether there are differences in the effects of c-tPCS at low-frequency non-

motor theta/delta (4 Hz) and motor-related high-frequency gamma (75 Hz) applied to the left M1 on CSE and motor performance. Given the importance of the frequency of the applied current, the pro-kinetic nature of gamma frequency, and the non-motor nature of theta frequency, we hypothesized that compared to c-tPCS at 4 Hz and sham, c-tPCS at 75 Hz would increase CSE. We postulated that changes in CSE would result from changes in intracortical inhibition and facilitation in the M1 area. Finally, we hypothesised that changes in CSE would lead to changes in motor performance.

2 MATERIAL AND METHODS

2. 1 Participants

Fifteen healthy volunteers (13 female) aged 18-35 (age 22.06 ±3.34 years) participated in this study. The study was approved by the Human Research Ethics Committee, Monash University, Australia. Written informed consent was obtained from all the participants according to the Declaration of Helsinki. All participants were right-handed, and none had contraindications to transcranial magnetic stimulation (TMS) as assessed by a TMS screening questionnaire (Keel et al., 2001). None of the participants had a history of any neurological disease and was not under any medications at the time of the experiment. The sample size was calculated (with a power of 80%) based on the data gathered from a pilot study (n=7).

2. 2 Experimental procedure

A randomized sham-controlled crossover design was implemented to assess the effect of single-session c-tPCS of left M1 for right FDI at 4 Hz and 75 Hz on CSE and motor performance. Participants attended three experimental sessions separated by at least one week, to avoid carry-over effects, and conducted at approximately the same time of the day (between 8 am – 12 pm) to avoid diurnal variation (Figure 2). In the experimental sessions, participants received: (i) c-tPCS at 4 Hz, (ii) c-tPCS at 75 Hz, and (iii) sham stimulation over left M1. The order of experimental conditions was counterbalanced across participants.

Single-pulse TMS induced motor evoked potentials (MEPs), paired-pulse induced intracortical facilitation (ICF) and short intracortical inhibition (SICI) in left M1 and motor performance using the groove pegboard test (GPT) were acquired before (baseline), immediately after (post 0) and 30 min after c-tPCS (post 30) using TMS at resting state.

“Please insert Figure 2 here”

2.3 Electromyography recording

During the experiment, participants were seated in a fully adjustable chair (treatment chair, MagVenture, Denmark) with their head and neck supported by a headrest, and their hand was resting on the armrest. Standard skin preparation was performed for each electrode site to achieve a low skin impedance of ⩽10kΩ (Gilmore and Meyers, 1983; Groppa et al., 2012). Electromyographic (EMG) activity was recorded from the right FDI muscle using pre-gelled self-adhesive Ag/AgCl electrodes arranged in a belly-tendon montage. EMG signals were recorded and amplified (x1000), with signals filtered (bandpass: 10-500 Hz), and then processed offline using commercially available software (LabChart™ software, AD Instruments, Australia) via a laboratory analog-digital interface (The PowerLab 4/35, AD Instruments, Australia).

2. 4 Assessment of corticospinal excitability

Single-pulse biphasic TMS stimuli were applied using MagPro R30 (MagOption, Denmark) stimulator (MagVenture, Denmark) connected via a figure-of-eight coil (max. initial dB/dt 28 KT/s near the coil surface). The coil was placed tangentially over the left hemisphere with the handle pointing backward at 45° from the midline sagittal plane of the skull (Vaseghi et al., 2015). The hotspot was identified as the area on the scalp where the largest and most stable MEPs could be obtained for the right FDI muscle.

The intensity for resting motor threshold (RMT) and the intensity that evoked 1mV peak-to-peak MEP amplitude (I_{1mv}) were determined prior to each c-tPCS session. The adaptive threshold hunting method based on the parametric estimation of sequential testing (PEST) was used to determine the TMS intensity related to RMT and I_{1mV} (Awiszus, 2003). A freeware program of TMS Motor Threshold Assessment Tool, MTAT 2.0 (https://www.clinicalresearcher.org/software.htm), which employs a maximum - likelihood PEST strategy without prior information, was used to assess RMT using the programs pre - determined TMS intensity (Awiszus and Borckardt, 2011). For RMT, an MEP amplitude of >50μV was considered a successful trial, and the MEP amplitude > 1mV was used as a successful trial for I_{1mV} (Silbert et al., 2013). When this intensity was not observed, a new intensity displayed by the program was used until the success point was reached. The target RMT and I_{1mV} were found when it was mathematically valid, and 95% confidence intervals

were within accuracy limits imposed by safety guidelines (Awiszus and Borckardt, 2011; Awiszus, 2012). Since the short-inter trial interval of TMS pulses is able to produce a hysteresis, whereby the MEP amplitude elicited by one TMS pulse can influence the MEP of the next TMS pulse, an inter-stimulus interval of 6s was used during threshold estimation and recording of MEP amplitudes (Moller et al., 2009; Julkunen et al., 2012).

Single-pulse and paired-pulse protocols were used in this study. The single-pulse protocol was performed with the TMS intensity adjusted to elicit 1mV peak-to-peak MEP amplitude at baseline to obtain 20 MEPs for the relaxed right FDI muscle, and this intensity was kept constant during post-intervention assessments.

Paired-pulse protocol was used for assessment of SICI and ICF in the M1 area. In this method, a sub-threshold TMS pulse for a motor response (conditioning stimulus) activates intracortical inhibitory circuits in the M1 area and changes the size of the MEP elicited by a suprathreshold TMS pulse (test stimulus). The TMS pulse was delivered (3ms) or (10ms) later to probe GABA-ergic or glutamatergic functions, respectively. The conditioning stimulus was set at 80% RMT (0.8 x RMT), and the test stimulus was set to elicit 1mV MEPs for both the SICI and ICF protocols.

Resting-state MEPs, SICI, and ICF were measured at baseline, immediately after (post 0) and 30 min after c-tPCS (post 30). Twenty trials per condition were applied in three blocks. These blocks were designed to deliver single and paired-pulse TMS randomly; each block contained 20 single, and 40 paired-pulse stimuli (20 ISI of 3ms and 20 ISI of 10ms).

2.5 Cathodal tPCS

2.5.1 Active stimulation

Cathodal transcranial pulsed current stimulation (Starstim, Neuroelectrics, Barcelona, Spain) was applied via a pair of circular shaped saline-soaked surface sponge electrodes (25 cm^2). The active electrode was placed over the TMS derived FDI hotspot of the left M1 and the return electrode was centred on the right supraorbital area (FP2, international 10-20 EEG system). The electrodes were fixed into a head cap (Neuroelectrics® Neoprene Head cap) and the head cap was tightened up by two pieces of Velcro strap. Another two pre-gelled self-adhesive electrodes were positioned behind the left ear (mastoid) as reference electrodes

(driven right leg (DRL) and common mode sense (CMS)). The impedance was kept at <5kΩ via the automatic impedance monitoring system of the device.

The c-tPCS waveform (Figure 1B) was unidirectional, rectangular pulses, with the stimulation parameters set by the system software (NIC v1.4 Windows), connected via cable to the electrodes. Two text files were created using MatLab and feed into the system software to generate c-tPCS at 4 Hz and 75 Hz. These stimulations were delivered with the parameters listed in table 1. The total charge was kept constant during both active stimulation sessions. The total charge for c-tPCS was calculated using the following formula:

$$TC = \frac{SI}{ES} \times PD \times NP$$

where TC is the total charge (milli-Coulomb per square centimetre, mC/cm^2), SI is the stimulus intensity (milli-Ampere, mA), ES is the electrode size (cm^2), PD is the pulse duration (msec), and NP is the number of pulses.

"Please insert Table 1 here"

2.5.2 Sham stimulation:

The sham stimulation was received for 50 seconds with the randomized order of either 4 Hz or 75 Hz. The current was ramped up and down over the first and last 10 seconds of stimulation with 30 seconds of stimulation at the target area. The current turned to zero mA in the remainder period (14:10 minutes) of the stimulation.

2.6 Double blinding

Both participants and the investigator were blinded to the type of stimulation. Two investigators were involved in the testing. The first investigator recorded all outcome measures but was blinded to the stimulation conditions. The second investigator applied the stimulation condition (4 Hz, 75 Hz, sham). Specifically, the participants were unaware of the stimulation condition they received. One of the challenges for the blinding in this study was the induction of light flashing sensations in the visual field (phosphenes) during low-frequency pulsed currents. Several publications report that tPCS induces phosphenes in the retina (Jaberzadeh et al., 2014; Jaberzadeh et al., 2015). Phosphenes are frequency-dependent, and lower frequencies are more likely to induce phosphenes than higher frequencies (Turi et

al., 2013). Indeed, at higher frequencies such as 75 Hz, light flash sensations are not noticed by the participants compared to lower frequencies such as 4 Hz. Therefore, an external source of flashing light with low frequency (4 Hz) was used to produce an illusion of the light flashing in all experimental conditions to blind the participants. This flashing light source was positioned in a place behind the participant while they received stimulation in all experimental sessions.

2.7 Measurement of side effects

All participants completed a questionnaire at the beginning, during, and following completion of interventions to rate the side effects such as paraesthesia, pain, and intensity of phosphenes perception. All participants ranked the unpleasantness of any scalp sensation using a numeric analogue scale (NAS) (e.g., 0: “no paraesthesia”, “no pain”, “no phosphenes”; 10: “worst paraesthesia”, “worst pain”, “strong phosphenes”). Moreover, the participants were asked to state whether they thought the stimulation they received was either active or sham at the end of each session.

2.8 MEP data management

The area under the curve of MEPs was quantified off-line from digitized averages of rectified EMG for conditioned and test stimuli in each trial via a custom-designed Powerlab 4/35 software. SICI and ICF were expressed as a ratio of mean conditioned MEP amplitude to the mean test MEP amplitude.

2.9 Measurement of motor performance

The GPT was used to test the motor performance (Lafayette instrument company, Model 32025). The test consists of a board with 25 keyholes that must be correctly rotated and inserted. The keys were placed on the keyholes by participants as fast as they could. The time required to complete all 25 keyholes was recorded in seconds/milliseconds, and this was considered as the outcome measure for evaluation of motor performance. The test was administered three-times for the dominant hand (GPT-R) and non-dominant hand (GPT-L) at all three-time points before and after the interventions. The average of three attempts was used in the analysis.

2.10 Statistical analysis

Statistical analyses were performed using SPSS software version 25.0 (IBM). Shapiro-Wilk test was used to test the normal distribution of the data. Log transformation was performed on the non-normally distributed data. If the data showed normal distribution after the log transformation, parametric tests were carried out. If the normal distribution were not corrected, and the skewness of the log data was more than one following log transformation, non-parametric tests were performed.

One-way analysis of variance (ANOVA) was used to assess the systematic differences between different experimental sessions at baseline for the outcome measure values of RMT, I_{1mv}, MEP amplitudes of I_{1mv}, SICI, ICF and time for completion of GPT (GPT-R and GPT-L). A two-way repeated-measures ANOVA (RM-ANOVA) was performed to assess the effects of c-tPCS on MEP at I_{1mv}, SICI, ICF and GPT (GPT-R and GPT-L) with the factor of group (4 Hz, 75 Hz, sham) and the factor of time (baseline, post 0 and post 30) as within-subject factors. Mauchly's test was used to test for assumption of sphericity, and Greenhouse-Geisser corrections were applied as necessary. In the case of significant effects, post hoc analyses with paired t-test (with Bonferroni correction) were applied. Pearson's correlation test was used to assess any correlation between the SICI/ICF and the CSE following c-tPCS.

A Friedman test was carried out on the mean values of paraesthesia, pain, and phosphenes perception to assess any significant difference between the groups for active and return electrodes. In addition, Pearson's chi-square test was used to assess whether participants were successfully blinded to the stimulation conditions (active or sham). The significance level for all tests was set at $p<0.05$. All data are presented as means and standard deviations unless otherwise indicated.

3 RESULTS

3.1 Comparison of baseline values

One-way ANOVA on baseline data were performed to ensure that there was no carry over effect and no systematic difference between groups. There was no difference in RMT and I_{1mv} between stimulation groups (RMT: $F_{(2,42)} = 0.075$, $p=0.928$; I_{1mV}: $F_{(2,42)} = 0.074$, $p = 0.929$) respectively. Further, there were no differences between groups in MEP amplitude resulting from any of the protocols performed at rest (I_{1mv}: $F_{(2,42)} = 0.964$, $p = 0.390$; SICI:

$F_{(2,42)}$ = 0.318, p = 0.730; ICF: $F_{(2,42)}$ = 0.203, p = 0.817; GPT-R: $F_{(2,42)}$ = 1.816, p = 0.175; GPT-L: $F_{(2,42)}$ = 0.655, p = 0.525).

3.2 Effect of c-tPCS on CSE

The effect of driving oscillatory activity on CSE, as reflected by single pulse TMS induced MEP amplitude at I_{1mv} was calculated. RM-ANOVA revealed significant main effect of group ($F_{(2,28)}$ = 8.689, p =0.004, η_p^2 = 0.383), time ($F_{(2,28)}$ = 11.180, p = 0.001, η_p^2 = 0.444) and significant group x time interaction ($F_{(4,56)}$ = 6.229, p=0.002, η_p^2 = 0.308) (Fig.3A, 4A, B, C).

Post hoc analysis demonstrated a significant increase in CSE with c-tPCS at 75 Hz (p=0.003) and 4 Hz (p=0.041) compared to sham. This was maintained at post 0 (75 Hz: $t_{(14)}$= -6.065, p<0.001; 4 Hz: $t_{(14)}$= -3.299, p=0.005) and at post 30 (75 Hz: $t_{(14)}$= -2.849, p=0.013). There were no difference between c-tPCS at 75 Hz and 4 Hz for CSE at post 0 ($t_{(14)}$ = 0.016, p=0.988), or post 30 ($t_{(14)}$ = -0.364, p=0.722). Within-session analyses revealed no difference between post 0 and post 30 for CSE of c-tPCS at 75 Hz ($t_{(14)}$ = 1.547, p=0.144). Similar to this, no difference was observed between CSE at post 0 and post 30 for c-tPCS at 4 Hz ($t_{(14)}$ = 1.358, p=0.196). There was no significant difference between baseline and post 0 for sham stimulation ($t_{(14)}$ = 0.806, p=0.433).

3.3 Effect of c-tPCS on SICI

The effect of c-tPCS on local $GABA_A$ inhibition reflected by SICI was investigated in this study. A RM-ANOVA showed a non-significant main effect of group ($F_{(2,28)}$ = 0.826, p=0.441, η_p^2 = 0.056), time ($F_{(2,28)}$ = 2.396, p=0.117, η_p^2 = 0.146) and a non-significant group x time interaction ($F_{(4,56)}$ = 1.158, p=0.338, η_p^2 = 0.076) (Fig.3B, 4D, E, F).

3.4 Effect of c-tPCS on ICF

The effect of c-tPCS on glutamate activity reflected by ICF was also investigated in this study. RM-ANOVA revealed a non-significant main effect of group ($F_{(2,28)}$ = 3.054, p=0.068,

$\eta_p^2 = 0.179$), significant time ($F_{(2,28)} = 8.429$, $p=0.004$, $\eta_p^2 = 0.376$) and a significant group x time interaction ($F_{(4,56)} = 3.923$, $p=0.014$, $\eta_p^2 = 0.219$) (Fig.3C, 4G, H, I).

Post-hoc analysis showed a significant increase in ICF following c-tPCS at 75 Hz compared with sham at post 0 ($t_{(14)} = 4.026$, $p=0.001$). Similarly, ICF at post 0 in c-tPCS with 4 Hz was significantly higher than at post 0 in sham ($t_{(14)} = 2.910$, $p = 0.011$). No difference was observed between ICF at baseline and post 0 in the sham session ($t_{(14)} = 0.351$, $p = 0.731$). Within group analysis showed that ICF at post 0 in c-tPCS with 75 Hz was significantly different from post 30 ($t_{(14)} = 2.843$, $p=0.013$). A significant difference was also observed between ICF at post 0 and post 30 in c-tPCS with 4 Hz ($t_{(14)} = 2.722$, $p=0.017$).

"Please insert figure 3 and 4 here"

3.5 Correlation between c-tPCS induced increase in CSE and increased ICF

Since the c-tPCS at both 4 Hz and 75 Hz significantly increase CSE and ICF, the correlation between these two phenomena were assessed. The TMS induced MEP and ICF at post 0 were used and the analysis detected a significant correlation between ICF and CSE in both c-tPCS at 4 Hz ($r = 0.602$, $p=0.018$) and 75 Hz ($r = 0.507$, $p = 0.054$) (Fig.5A, B).

"Please insert figure 5 here"

3.6 Effect of c-tPCS on motor performance

The effect of c-tPCS on motor performance on both dominant (GPT-R) and non-dominant (GPT-L) was investigated in this study. The RM-ANOVA on GPT-L revealed no significant effect of group ($F_{(2,28)GPT\text{-}L} = 1.938$, $p=0.166$, $\eta_p^2 = 0.122$), time ($F_{(2,28)GPT\text{-}L} = 1.933$, $p=0.178$, η_p^2,= 0.121) or group x time interaction ($F_{(4,56)GPT\text{-}L} = 0.008$, $p=0.996$, $\eta_p^2 = 0.001$). For the GPT-R, there was a significant effect of group ($F_{(2,28)GPT\text{-}R} = 4.285$, $p=0.036$, $\eta_p^2 = 0.234$), but no significant effect of time ($F_{(2,28)GPT\text{-}R} = 1.322$, $p=0.280$, $\eta_p^2 = 0.086$) or group x time interaction ($F_{(2,28)GPT\text{-}R} = 0.399$, $p=0.729$, $\eta_p^2 = 0.028$) (Fig.6A and B). Post-hoc analysis of the factor of group for GPT-R showed that there is a significant higher motor performance with c-tPCS at 4 ($p=0.043$) and 75 Hz ($p=0.035$) compared to sham.

"Please insert figure 6 here"

3.7 Participant reported experience during c-tPCS

Participants' experiences on stimulation such as paraesthesia, pain and phosphenes were recorded prior, during and after the completion of the intervention. There was no difference in the perception of paraesthesia and pain between the three stimulation conditions (4 Hz, 75 Hz and sham) under both active and return electrodes ($Paraesthesia_{active}$: $X^2(2) = 5.245$, $p=0.073$; $pain_{active}$: $X^2(2) = 3.000$, $p=0.223$; $paraesthesia_{return}$: $X^2(2) = 3.200$, $p=0.202$; $pain_{return}$: $X^2(2) = 2.000$, $p=0.368$). Moreover, there was no significant difference in the strength of phosphenes perceived between the three stimulation conditions ($X^2(2) = 0.065$, $p=0.968$). Overall c-tPCS was safe and tolerable with negligible side effects.

The participant's judgement on the stimulation conditions assessed with Pearson's chi-squared showed no significant differences between active and sham conditions ($X^2(2) = 2.500$, $p=0.287$), demonstrating that participants were not able to determine the type of stimulation.

4 DISCUSSION

To the best of our knowledge, this is the first study that attempted to investigate the effect of a single session monophasic c-tPCS at 4 Hz and 75 Hz over the left M1 on CSE and motor performance in healthy young individuals. The findings of the current study demonstrate that c-tPCS at both 4 Hz and 75 Hz significantly increase the CSE measured by TMS induced MEPs compared to sham. In addition, the results show a significant increase only in glutamate activity as measured by ICF, but not in the $GABA_A$ activity as measured by SICI. The ICF effect correlates with the CSE effects induced by c-tPCS at 4 Hz but not 75 Hz. The motor performance remained unchanged following the stimulation. Lastly, participants were successfully blinded to the active and sham stimulation conditions using light flashing source, and no significant differences between groups were found for pain, paraesthesia, and phosphenes.

4.1 Effect of c-tPCS on CSE

We hypothesised that, compared to sham and c-tPCS at 4 Hz, monophasic c-tPCS at 75 Hz would induce a significant increase in CSE in healthy individuals. This hypothesis was partially supported by the findings in current study. We found a significant increase in CSE in both frequencies of 4 and 75 Hz compared to sham and there was no significant difference between the active stimulation conditions. In a similar study applying low-frequency c-tPCS (i.e.0.5 Hz) on M1 in high-definition montage has reported a reduction in CSE (Luu et al., 2016). The authors concluded that low-frequency c-tPCS current produces inhibition. However, the electrophysiological evidence indicates that theta phase neural oscillations can modulate the amplitude of gamma neural oscillations via theta-gamma cross-frequency coupling (Canolty et al., 2006; Demiralp et al., 2007). In light of this concept, a review on tACS mechanism propose that the electrical stimulation of the cortex at theta range frequency would induce theta neural oscillation and this would then modulate the gamma neural oscillation (Herrmann et al., 2013). Accordingly, tACS at slower theta frequency of 4 Hz has indicated to integrate higher number of gamma cycles per theta cycle and enhance working memory capacity compared to higher theta frequency of 7 Hz that resulted reduction in working memory capacity (Wolinski et al., 2018). As neuromodulation techniques, tACS and tPCS shares similar characteristics, the observed scale up of CSE following 4 Hz is supposed to be occurred in line with the theta-gamma cross-frequency coupling theory. One possible explanation might be that c-tPCS at 4 Hz induced theta neural oscillation and it has then induced the gamma neural oscillation that led to an increase in CSE. On the other hand, the observed increase in CSE following c-tPCS at 75 Hz would be due to the associated pro-kinetic nature of gamma frequency.

In general, the exact mechanism underpinning the effect of tPCS is unknown. Previous studies have reported that bidirectional tPCS may entrain cortical oscillation (Datta et al., 2013; Castillo Saavedra et al., 2014; Morales-Quezada et al., 2015). It should be noted that the present study used c-tPCS with unidirectional negative pulses, which produce negative NDCC (hyperpolarization only). However, a tACS study has shown that the application of negative half-sign wave can also entrain the network similar to full-sign wave (Ali et al., 2013). Therefore, in line with the findings in tACS studies, the effects of c-tPCS may be attributed to the entrainment whereas the after-effects of stimulation may be attributed to the spike-timing-dependent plasticity (STDP) or long-term potentiation (LTP) (Thut et al., 2011; Helfrich et al., 2014; Veniero et al., 2015). Since the present study assessed the after-effects of stimulation, the c-tPCS induced effect may rather rely on the STDP/LTP. According to this

mechanism, synapses are either strengthened or weakened based on the exact timing of the input or output activity (Zaehle et al., 2010). When the pre-synaptic activity precedes a post-synaptic activity, the synapses are strengthened, and LTP is induced. In contrast, the post-synaptic activity precedes a pre-synaptic activity; the synapses are weakened and occurs long-term depression (LTD) (Zaehle et al., 2010). The gamma oscillation is thought to provide timing necessary for STDP. In support of this notion, previous experiments on animals have demonstrated that gamma oscillations are actively engaged in the production of LTP-like plasticity (Diba and Buzsaki, 2007; Izaki and Akema, 2008; Girardeau et al., 2009; Papazachariadis et al., 2014). However, slow oscillation type like theta has not been identified to provide timing for STDP (Nyhus and Curran, 2010). Instead, the literature suggests that theta oscillation cause depolarization, which leads to the opening of NMDA channels, causing calcium influx initiating the synaptic modification process to produce LTP. Given the importance of the frequency of stimulation, both 4 and 75 Hz may have acted to entrain neuronal population in M1 with repeated pulses, which are more susceptible to gamma and theta frequencies, thereby causing to strengthen the synapse where LTP like plasticity can occur to induce increase in CSE.

The similar findings of 4 and 75 Hz are also supported by the studies showed an increase in CSE with high intensity (1.5-2 mA) c-tDCS induced homeostatic plasticity (Batsikadze et al., 2013; Moliadze et al., 2015; Jamil et al., 2016). Accordingly, it is plausible to interpret that c-tPCS fundamentally produce inhibition that relies on the negative NDCC induced via polarity-dependent effect of c-tPCS, but the overexcitation due to the high intensity may have reversed the effects. This might be due to the dependency of the plasticity change on the amount of neuronal calcium influx by different stimulation protocols (Batsikadze et al., 2013; Moliadze et al., 2015; Jamil et al., 2016). This proposition is supported by the fact that low postsynaptic calcium enhancement causes LTD, whereas high postsynaptic calcium produces LTP (Cho et al., 2001; Lisman, 2001). Therefore, the high stimulation intensity, e.g., 1.5 mA c-tPCS, might increase calcium level and induce LTP like plasticity. In addition, the current applied to the scalp via conventional electrode montage can affect several regions of the brain by involving areas beyond the target area (Datta et al., 2009; Polania et al., 2011; Polania et al., 2011). Consequently, high-intensity c-tPCS may have increased the electric field over the target brain region and recruited other non-target brain regions that could indirectly change the direction of neural plasticity in the target brain area.

4.2 Effect of c-tPCS on SICI and ICF

We hypothesized that changes in CSE would be related to changes in SICI and ICF. The findings partially support this hypothesis as a significant increase in ICF was observed, while SICI remained unaffected by the stimulation. Given that glutamate and NMDA receptors are involved in mediating ICF (Ziemann et al., 1996; Chen et al., 1998), we conclude that glutamatergic and NMDA receptor function has been up-regulated following monophasic c-tPCS at both 4 and 75 Hz. The increase ICF was correlated with the CSE effect produced by both c-tPCS at 4 and 75 Hz by showing the effect of ICF on CSE.

4.3 Effect of c-tPCS on motor performance

We further hypothesised that the changes in CSE could affect motor performance. This was not supported by the findings on motor performance in the present study. Despite the observed significant effect on CSE for the c-tPCS at 4 Hz and 75 Hz, no improvement was observed in motor performance. It indicates that c-tPCS significantly modulates CSE, but the changes do not necessarily translate into a significant change in motor performance in healthy individuals. On the other hand, the state-dependent effect (resting vs. task performance) may have contributed to the observed no change in motor performance. Previous evidence on tDCS and tACS have reported the relationship between cognitive brain state and the stimulation effects (Feurra et al., 2013; Neuling et al., 2013; Gill et al., 2015; Hsu et al., 2016; Violante et al., 2017). For example, a study reported an increase in MEP when anodal tDCS was given at rest though it was decreased when it is applied during a task (Antal et al., 2007). Similarly, the change of the cognitive state from resting (MEP recordings) to the active when they involve in a motor task may have involved with no change in motor performance. At present, there are no available studies that have assessed the state-dependent effect of c-tPCS in 4 Hz and 75 Hz. In addition, the participants only received c-tPCS during one session and a single session c-tPCS may be insufficient to show a significant effect in motor performance, as the participants in this study were healthy and without any impairment.

4.4 Participant reported side effects during c-tPCS

In general, the use of c-tPCS showed minimal/no side effects among healthy participants. Moreover, participants were successfully blinded to the stimulation condition, and they were unable to distinguish whether the stimulation was real or sham. It has been suggested that the

side effects of paraesthesia would be due to the electrochemical effect of NDCC under the electrodes (Durand et al., 2002; Dundas et al., 2007; Palm et al., 2008). However, no studies that used c-tPCS to compare these effects directly, and future studies should be targeted to investigate these effects based on c-tPCS.

The effect of phosphenes perception on the retina was not significantly different between the 4 Hz, 75 Hz, and sham conditions. There are, however, no studies that have assessed the phosphenes effect from c-tPCS. The evidence from tACS studies has shown that low-frequency stimulation produces noticeably more phosphenes compared to higher frequencies (Turi et al., 2013). Further, the current intensity above 1 mA and the distance of the electrodes to the eye are also likely to induce phosphenes (Schwiedrzik, 2009; Turi et al., 2013; Fertonani et al., 2015). Hence, the observed phosphenes effect is likely to be related to both the frequency of stimulation and the intensity of the applied current (1.5 mA). Nevertheless, the flashing light source successfully blinded the participants to phosphenes and did not confound the observed CSE changes. The flashing light method could also be used in tPCS studies with different frequencies and for frequency-dependent tACS studies that produce phosphenes on the retina.

4.5 Limitations

The findings of this study have to be seen in the light of some limitations. The involvement of healthy young participants (less than 35 years old), may limit the generalizability of the results to older and individuals with neurological disorders. Another limitation is the fact that the majority of the participants in this study were females (82.3%). Importantly previous studies have shown that female sex hormones have an impact on the CSE changes (Smith et al., 1999; Inghilleri et al., 2004; Hattemer et al., 2007). We did not account the stage of the menstrual cycle in this study, which may have affected the results. We did not assess gender as a confounding variable, as the majority of the participants were female. Therefore, cautions should be taken when extrapolating the findings to the male population. In addition, a study by Peurala et al. (2008) has shown that SICI can be contaminated by short-interval intracortical facilitation (SICF) at ISI of 2-3 ms with the conditioning stimulus at 95% active motor threshold. Since the use of 80% RMT in the present study can correspond to the AMT range used by Peurala et al. (2008), the contamination SICI from SICF would be high in the present study though we did not see any significant effect in SICI. Finally, we did not

incorporate a navigation system in this study. A study by Jung et al. (2010) has shown that there is no significant difference in the reproducibility and variability of MEPs with non-navigated compared to optically tracked TMS navigation system (Jung et al., 2010). On the other hand, few studies have shown that MEP recording could be varied whether navigation is used ((Julkunen et al., 2009; Cincotta et al., 2010). As such, we believed that the reproducibility of MEP recordings might have affected by non-navigated TMS.

4.6 Suggestions for future research

Future studies are required to assess the effect beyond 30 min post-stimulation. Since the after-effects did not return to baseline by the end of the testing period, we were unable to estimate the lasting of offline effects. In addition, assessing the effect of c-tPCS at 4 Hz and 75 Hz on brain electrical activity using EEG could aid in understanding the mechanism of entrainment. Furthermore, future studies should account for the effects of gender, age, menstrual time and genotype on CSE following c-tPCS. Finally, the assessment of different frequencies of c-tPCS should help to identify the frequency-specific effect of this emerging novel technique.

5 CONCLUSIONS

Our findings indicate that c-tPCS at 4 Hz and 75 Hz leads to a significant increase in corticospinal excitability that lasts for 30 min post-stimulation compared to sham. Cathodal-tPCS at 4 Hz and 75 Hz produces an excitatory effect. Moreover, this observed effect of c-tPCS at 4 Hz was correlated with intracortical facilitation. In addition, the motor performance did not show any changes following stimulation.

Figure legends

Figure 1. Transcranial pulsed current stimulation (tPCS) techniques, (a). A-tPCS (unidirectional positive pulses) (b). C-tPCS (unidirectional negative pulses) (d). tPCS (bidirectional pulses)

Figure 2. (A). Experimental set-up of the double blind randomized controlled cross-over design. (B). Bipolar montage (anode: red; cathode: blue). (C). Normal electric field map for the bipolar montage. Baseline and post measurements consisted of 20 blocks of 3 TMS pulses: MEP, SICI and ICF, and GPT. c-tPCS: cathodal transcranial pulsed current stimulation; MEP: motor evoked potentials; SICI: short-interval intracortical inhibition; ICF: intracortical facilitation; GPT: grooved peg board test

Figure 3. The effect of c-tPCS on CSE. Averaged MEP amplitude (±SEM) values (A), SICI (B), and ICF (C) at baseline, post 0 (immediately after c-tPCS) and post 30 (30 min after c-tPCS): 4 Hz, 75 Hz, and sham. Filled symbols indicate significant deviation of the post-intervention MEP amplitude, SICI and ICF compared to the baseline. MEP: motor evoked potentials; SICI: short-interval intracortical inhibition; ICF: intracortical facilitation. *: <0.01. Data are reported as mean ± SEM.

Figure 4. Individual participant data. MEP amplitude for 4 Hz (A), 75 Hz (B), sham (C); SICI for 4 Hz (D), 75 Hz (E), sham (F) and ICF for 4 Hz (G), 75 Hz (H), sham (I)

Figure 5. Correlation between CSE and ICF at post 0 for 4 Hz (A) and 75 Hz (B)

Figure 6. The effect of c-tPCS on motor performance. (A) GPT-R (B) GPT-L. GPT-R: grooved pegboard test-right hand; GPT-L: groove pegboard test-left hand. Data are reported as mean ± SEM.

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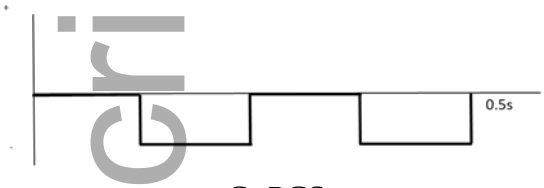
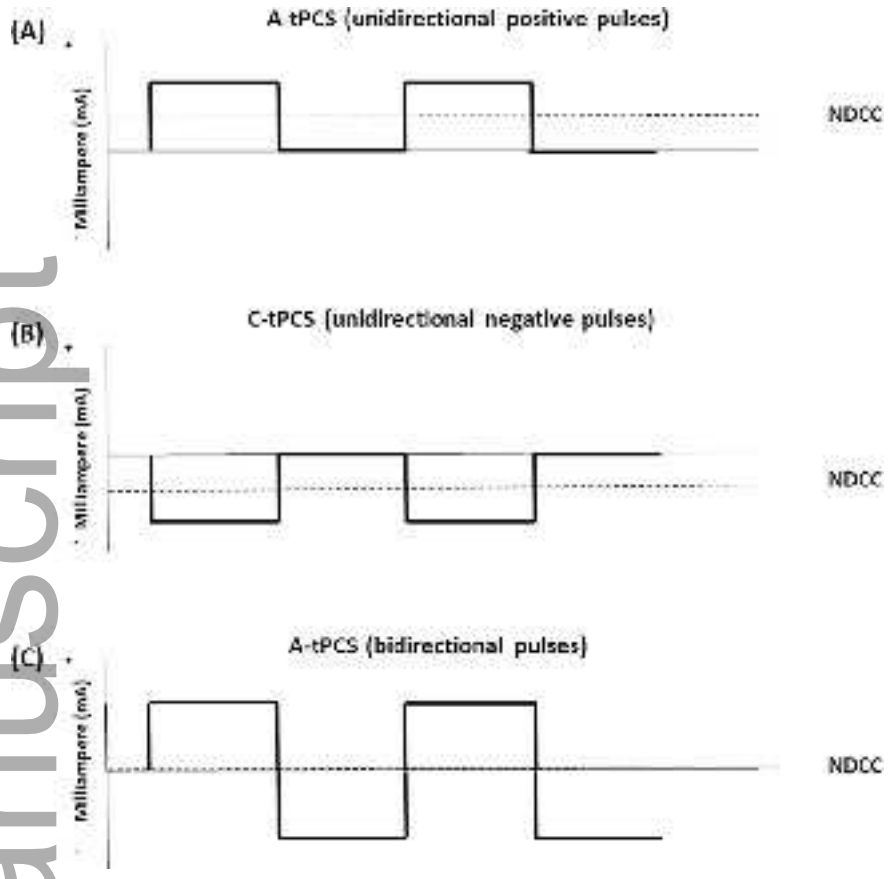
Intervention	Current intensity (mA)	Pulse duration (ms)	Inter-pulse interval (ms)	Total duration (min)
 C-tPCS$_{4Hz}$	1.5	125	125	15



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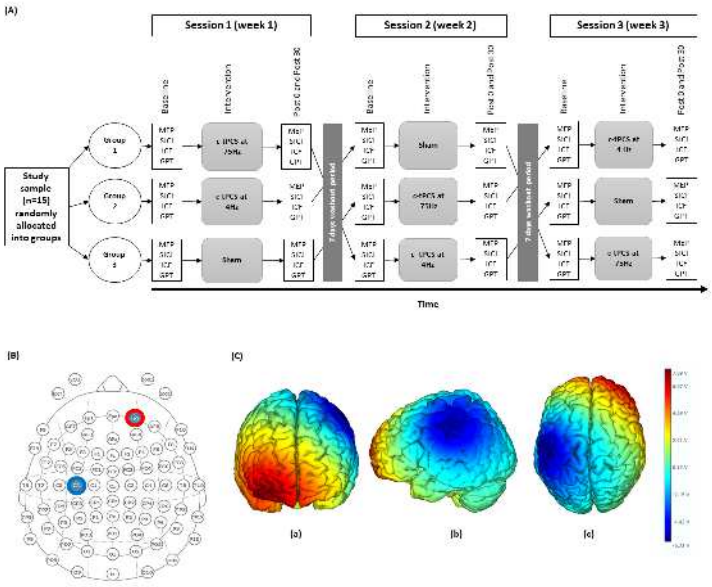

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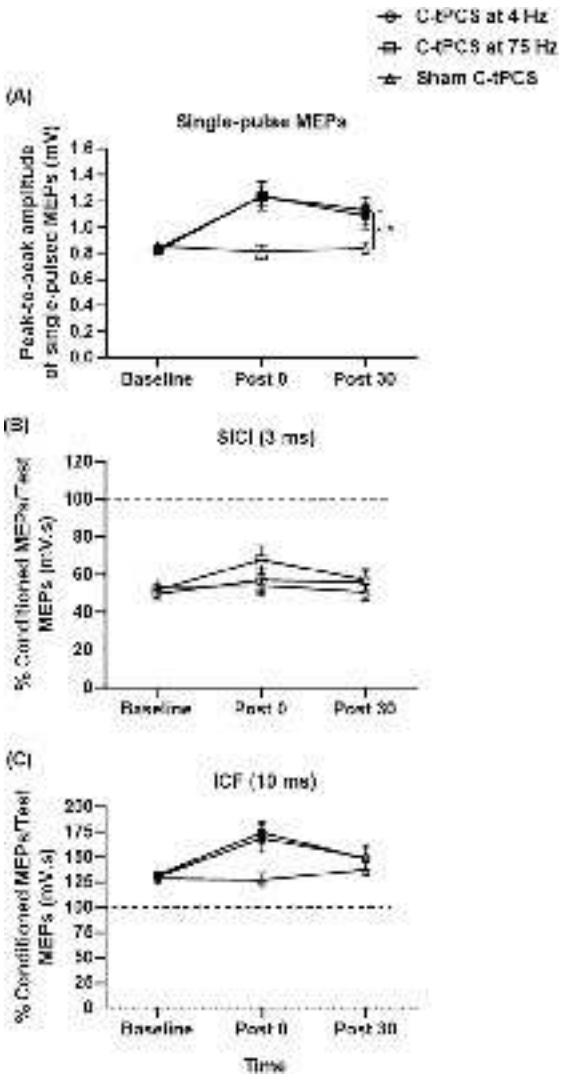

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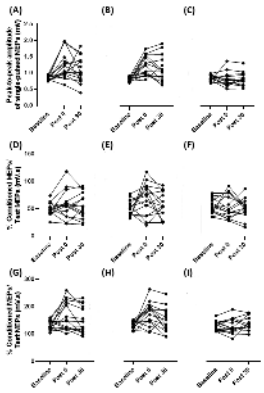

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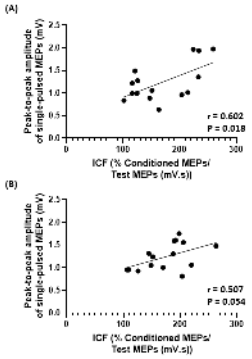

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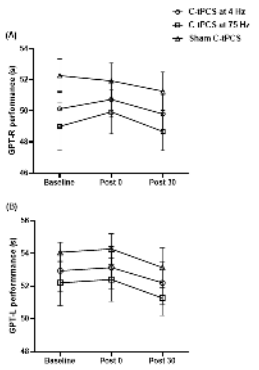

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Title:
The effects of a single-session cathodal transcranial pulsed current stimulation on corticospinal excitability: A randomized sham-controlled double-blinded study

Date:
2020-12

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