**Title:** Comparing the effects of anodal and cathodal transcranial direct current stimulation of primary motor cortex at varying intensities on motor learning in healthy young adults

**Abstract:**

Inconsistent results are observed in the effects of transcranial direct current stimulation (tDCS) with different montages on motor learning. This study aimed to compare the effects of anodal and cathodal tDCS over primary motor cortex (M1) at different intensities (1 and 2 mA) on motor learning in healthy young adults. The participants were randomly divided to five groups: 1) 1mA M1 c-tDCS, 2) 1mA M1 a-tDCS, 3) 2 mA M1 c-tDCS, 4) 2 mA M1 a-tDCS and 5) M1 sham tDCS. The groups received 20-minute stimulation concurrent with serial response time test (SRTT) implicitly, while the tDCS was turned off after 30 seconds in the sham tDCS group. Response time (RT) and error rate (ER) during SRTT were assessed prior, during and 72 hours after the intervention. The results indicated that online learning occurred in all groups (P < 0.05), except in M1 c-tDCS (1 mA) (P>0.05). In addition, offline learning was observed in 1 mA M1 a-tDCS, 2mA M1 a-tDCS and 2 mA M1 c-tDCS as compared to sham tDCS and M1 c-tDCS (1 mA) groups (P < 0.05). On the other hand, 1 mA M1 c-tDCS group did not indicate any consolidation effect and even a trend toward negative offline learning. M1 a-tDCS with different intensities and also 2 mA M1 c-tDCS may be helpful for the enhancement of motor learning in young adults. Considering the deterioration effect of 1 mA M1 c-tDCS, it seems that caution should be applied in using it to improve motor learning.

**Key words:** Motor learning, Anodal tDCS, Cathodal tDCS, Stimulation intensity, Primary motor cortex, Serial response time test

**Introduction:**

Engaging in repetitive performance practice has been associated with the enhancement of neuro-motor adaptations, functional abilities, and overall performance improvement, as well as motor learning (Jackson *et al.*, 2019; Meek *et al.*, 2021; Parma *et al.*, 2021). Enhancing motor learning to facilitate skill acquisition and memory consolidation represents a current challenge in both skill acquisition and functional rehabilitation domains (Debarnot *et al.*, 2019).

Serial Reaction Time Tasks (SRTT) are widely utilized for evaluating implicit sequence motor learning (Trofimova *et al.*, 2020). This involves the observation of repeated sequences or random events during task performance and is recognized as the most commonly used test for this purpose (Debarnot *et al.*, 2019). Research has indicated neuroplastic changes during implicit motor learning across various brain regions in young, healthy adults (Debarnot *et al.*, 2019). Presently, non-invasive brain stimulation (NIBS) techniques are employed to modulate motor and cognitive functions, aiding the learning process in both healthy adults and individuals with neurological conditions (Buch *et al.*, 2017; Lefebvre *et al.*, 2017; Cole *et al.*, 2018; Santos *et al.*, 2020)

Research indicates that tDCS has the capacity to impact resting membrane potential, potentially leading to an increase or decrease in its levels (Nitsche & Paulus, 2000; Nitsche *et al.*, 2008). The direction of this influence depends on whether anodal or cathodal tDCS is administered (Lefebvre *et al.*, 2017; Cole *et al.*, 2018; Santos *et al.*, 2020). Notably, research indicates that combining M1 anodal tDCS with motor training can lead to improved motor performance and learning compared to cathodal tDCS and sham stimulation (Stagg *et al.*, 2009; Ostry & Gribble, 2016; Karok *et al.*, 2017; Spampinato & Celnik, 2018; Debarnot *et al.*, 2019; Talimkhani *et al.*, 2019; Iannone *et al.*, 2022). Conversely, a study found that applying cerebellar a-tDCS reduced motor learning during SRTT (Jongkees *et al.*, 2019). Some studies have demonstrated that c-tDCS can enhance motor learning in SRTT (Greeley *et al.*, 2020; Pollok *et al.*, 2021). Furthermore, certain studies have highlighted the positive effects of both a-tDCS and c-tDCS over M1 compared to sham tDCS on motor learning (Ciechanski & Kirton, 2017; Shilo & Lavidor, 2019).

Evidence suggests that the intensity of tDCS, regardless of polarity, can significantly impact the outcomes of tDCS (Stagg *et al.*, 2011; Ciechanski & Kirton, 2017; Greeley *et al.*, 2020). Some studies have shown that both 1 mA and 2 mA tDCS can improve motor learning compared to sham conditions in healthy adults (Stagg *et al.*, 2009; Ciechanski & Kirton, 2017; Shilo & Lavidor, 2019). Conversely, other studies have indicated that 1 mA or 2 mA tDCS may interfere with the motor learning process compared to sham stimulation (Stagg *et al.*, 2011; Greeley *et al.*, 2020).

It appears that previous studies have presented conflicting findings regarding the efficacy of tDCS with different polarities and intensities on motor learning (Stagg *et al.*, 2011; Ciechanski & Kirton, 2017; Buchwald *et al.*, 2019; Shilo & Lavidor, 2019). However, it is essential to determine the most effective approach to optimize the use of tDCS for enhancing motor learning. Interestingly, there is a lack of research comparing the effects of different polarities and intensities of tDCS on motor learning in healthy young adults. This study aims to explore the impacts of tDCS on M1 at varying intensities and polarities on motor learning in healthy young adults using the SRTT. The hypothesis for this study is:

- Concurrent application of 1 and 2 mA M1 a-tDCS and SRTT would reduce RT and ER during and after application of interventions compared to concurrent sham a-tDCS and SRTT.

- Concurrent application of 1 and 2 mA M1 c-tDCS and SRTT would have no effect on reduction of RT and ER during and after completion of interventions compared to sham tDCS and SRTT.

- Concurrent application of 1 mA M1 a-tDCS and SRTT would reduce RT and ER during and after completion of interventions compared to 1 mA M1 c-tDCS and SRTT.

- Concurrent application of 2 mA M1 a-tDCS and SRTT would reduce RT and ER during and after completion of interventions compared to 2 mA M1 c-tDCS and SRTT.

**Method and materials:**

**Participants**

In this study, the sample size of 77 was determined using G Power software, aiming for a 95% confidence level and 85% power, according to a similar study (Ciechanski & Kirton, 2017). The participants were included in the study if they were: 1) healthy, 2) right-handed and 3) aged between 18 and 35 years. The participants who: 1) reporting any history of neurological diseases such as Parkinson's, Alzheimer's, schizophrenia and dyslexia, 2) having psychological diseases, 3) receiving any brain stimulation affecting the central nervous system during the last two weeks, 4) having severe perceptual or memory problems (scores of less than 21, assessed by Mini-Mental Status Examination (MMSE)), 5) reporting the use of any sedative drugs in the last two days, 6) the presence of any symptoms of movement disorders in the upper limb of the right hand, 7) the presence of any symptoms of radiculopathy, carpal tunnel syndrome in the right hand as diagnosed by the researcher, 8) having visual and hearing dysfunction, 9) reporting dizziness, 10) alcoholism and 11) having heart rate regulator users were excluded from the study. In this study, 100 right-handed young and healthy volunteers were invited to assess for eligibility based on inclusion and exclusion criteria. Based on the inclusion and exclusion criteria of the study, 20 volunteers were excluded from the study and 80 healthy individuals included in the study. The remaining participants were randomly assigned to one of the five groups by computerized random number generator: 1) M1 c-tDCS with an intensity of 1 mA, 2) M1 a-tDCS with an intensity of 1 mA, 3) M1 c-tDCS with an intensity of 2 mA, 4) M1 a-tDCS with an intensity of 2 mA and 5) M1 sham tDCS. Finally, 77 participants completed the entire study, and the data gathered from these individuals underwent analysis.

This study was approved by the Human Ethics Committee of the XXX (IR.SEMUMS.REC.1401.284) and registered as a clinical trial on the XXX (The registration number is IRCT2022102305677N2). The current study was performed during the first half of 2023 in the Neuromuscular Rehabilitation Research Center. A written informed consent was signed by all participants before participation in the study. This study met the CONSORT checklist criteria.

<<<Please insert Figure 1 here>>>

**Study design**

This study employed a randomized double-blind clinical trial with a parallel design. Concurrent with the application of active or sham M1 tDCS, the participants in all five groups were asked to perform the SRTT training.

**Tool for induction and assessment of motor learning**

In this study, the Color Matching Test (CMT), a custom-designed software program, was used to induce and assess both online and offline motor learning. The CMT is designed to simulate an SRTT condition with a second-order structural pattern. SRTT is one of the most used methods to assess implicit motor learning (Masoudian *et al.*, 2020). The participants were instructed to perform the SRTT using the index finger of their dominant hand (right hand) in the shortest possible time, without informing the participants about the pattern of the stimuli. The CMT software presents colored squares on a computer screen, with four possible colors: yellow, green, red, and blue. Each color is associated with a specific key on the computer keyboard (yellow - right shift key, red - C, green - M, blue - left shift key). By pressing the correct key, the next colored square appears.

The participants completed two pre-test blocks, followed by 8 blocks as the main training task 30 minutes after the pre-test, and finally two blocks 72 hours after the completion of the main training, serving as a retention test. The second block of the pre-test was considered as the baseline data. Except for blocks 7 and 8, which were presented randomly, all other blocks followed an ordered pattern (Figure 2). Each ordered block consisted of 10 trials, with each trial containing 8 color cues in a specific sequence (e.g., Yellow-Red-Green-Yellow-Blue-Green-Yellow-Green...) (Figure 2). The duration of the SRTT was depended on the response speed of each participant. However, there was considered a 30-second rest between each block of SRTT in the CMT program.

The RT of each sequence and the ER were measured before, during and 72 hours after applying the intervention. The RT was measured as the mean completion time of each block in each test. In addition, the ER was obtained from the mean errors of each block in each test. Online learning (within session) is defined as any reduction in the RT and/or ER at block 10 (Train 10) compared to block 3 (Train 3). In addition, offline learning, as the lasting effect of learning, was considered after the completing of the training (between sessions). Accordingly, offline learning was defined as any decrease in the RT or ER of block 12 (Post 12) compared to block 2 (Pre 2). Furthermore, the consolidation effect of learning was defined as any reduction or maintenance of RT or ER in block 12 (Post 12) compared to block 10 (Train 10), as illustrated in Figure 2.

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**Transcranial direct current stimulation**

In the current study, tDCS device (ActivaDose® II, ActivaTeKTM Inc., Gilroy, CA, USA) was used to apply direct current intensities of 1 and 2 mA for 20 min concurrent with SRTT training. The current intensity was controlled by the ammeter of the device. The size of the stimulation electrodes was 5×7 cm. The electrodes were carbon rubber electrodes. The current ramp-up and down during a period of 10 seconds of the beginning and the end of stimulation.

In two M1 a-tDCS groups, the active anode electrode was placed over the left M1 (C3, International 10–20 system) and the cathode return electrode was placed over the right side of the contralateral supraorbital area, respectively. In the two c-tDCS groups, the active cathode electrode was located over the left M1 (C3, International 10–20 system) and the return anode electrode was located over the right side of the contralateral supraorbital area. The electrodes were placed transversely over left M1 area and right supraorbital region. Electrical stimulation was administered to either the M1 a-tDCS or M1 c-tDCS groups at an intensity of 1 mA, while the remaining groups received stimulation at an intensity of 2 mA.

In the Sham tDCS group, electrodes labeled as anode and cathode, along with a stimulation intensity of 1 mA or 2 mA, were randomly chosen and placed over the designated areas for a duration of 20 minutes. The stimulation was gradually deactivated after 30 seconds, following the Fade-in Short Stimulation Fade-out (FiSsFo) approach. Nonetheless, the electrode montage mirrored that of the active M1 tDCS groups.

**Experimental procedures**

Participants were asked to sit in front of a computer monitor and press the correct key when they saw a square on the monitor. Concurrent with the main SRTT test, participants also received the tDCS intervention corresponding to their group (Hardwick & Celnik, 2014). Several studies have suggested that simultaneous application of tDCS and SRTT can significantly enhance motor behavior (Hanning *et al.*, 2023). Therefore, in the present study, the tDCS intervention was administered concurrently with SRTT (Figure 2).

Two researchers were involved in this study. The first one was responsible for pre- and post-intervention assessments of outcome measures, who was blinded to the participant’s grouping. The second researcher was responsible for applying the interventions. Participants were fully unaware of the interventions in the other groups, and they were also kept blind to whether they were receiving sham or active tDCS sessions. To evaluate the integrity of blinding, participants were asked to guess the type of intervention (active or sham) after each session. Their responses regarding the nature of the tDCS intervention they received were then compared for the active and sham conditions using a Pearson's chi-squared test (χ2) to assess blinding integrity.

**Assessment of the side effects**

To assess the side or adverse effects, all participants were asked to report any discomfort such as tingling, itching, burning, headache, dizziness, heat, electric shock, etc, during or after the intervention in each session by completing the numeric analogue scales (NAS) questionnaire (Nitsche *et al.*, 2008; George & Aston-Jones, 2010).

**Data analysis**

This study employed SPSS version 22 software for data analysis. Shapiro-wilk test was used to check the normal distribution of data. Normal distribution was revealed for all variables in the groups. To test the lack of differences in the baseline values among groups, a one-way ANOVA was used. A general linear repeated measure ANOVA was conducted to assess the main effects of “Group”, “Time” (Pre 2, Train 3, Train 10, and Post 12), and their interactions on the RT and ER. In addition, to compare online and offline learning and also the consolidation effect of learning among groups, a one-way ANOVA was conducted. Post hoc tests with Bonferroni correction were also done. Type I error (α) was set at 0.05 and the power of tests was determined at 0.85.

**Results:**

The demographic details and baseline data for the participants in each group are listed in Table 1. There were no significant differences among groups in age, gender, MMSE, and baseline RT and ER (Pre 2) (P > 0.05).

<<<Please insert Table 1 here>>>

Table 2 presents the results of general linear repeated measures ANOVA. The between-subjects main effect of “Group” and the within-subjects main effect of “Time” were significant for both RT and ER (P < 0.001). The analysis also showed that there was a significant interaction effect between “Group” and “Time” (P < 0.001) for both RT and ER (Table 2).

<<<Please insert Table 2 here>>>

Post hoc paired T-test using Bonferroni correction showed significant reduction in RT between Train3 and Train10, between Pre 2 and Post 12 in M1 a-tDCS (1mA), M1 a-tDCS (2mA), M1 c-tDCS (2 mA) and sham groups (P < 0.01), which indicated online improvement and offline learning (Figure 3). In addition, post hoc analysis indicated that the amount of decreasing RT of Train 10, as online learning, in M1 a-tDCS (1 mA) and M1 a-tDCS (2mA) groups was more than M1 c-tDCS (2 mA) and sham groups (P<0.01, Figure 4, A). However, a significant reduction in ER between Train 3 and Train 10 and between Pre 2 and Post 12 was only shown in M1 a-tDCS (1mA) and M1 a-tDCS (2mA) groups (P<0.001) (Figure 3). Moreover, one-way ANOVA analysis indicated that there were significant differences in online and offline learning (RT and ER reduction) among groups (P < 0.001, Table 3).

Figure 4 also indicates more offline learning, based on RT reduction, in M1 a-tDCS (1mA) than M1 c-tDCS (1mA) (p=0.01) and sham M1 a-tDCS groups (p=0.02). There was more offline learning, based on ER reduction, in M1 a-tDCS (1 mA), M1 a-tDCS (2mA) and M1 c-tDCS (2 mA) groups as compared to M1 c-tDCS (1mA) and sham M1 a-tDCS groups (p<0.03). In contrast, there were no significant decreases in RT and ER between Train 3 and Train 10 (P>0.05) and Pre 2 and Post 2 (P>0.05) in M1 c-tDCS (1 mA) group, which indicated deficits in online and offline learning (Figure 3, 4).

In addition, there was a consolidation effect of learning in all groups, except in M1 c-tDCS (1 mA) group (Figure 4). The results show a reduction in RT and ER, which lasted and even shows a trend toward more reduction offline, between Train10 and Post2 in the M1 a-tDCS (2mA) and M1 c-tDCS (2 mA) groups (fig. 4 a, b). On the other hand, the M1 c-tDCS (1 mA) group did not show any offline effect and even showed a trend toward negative offline learning (comparison between Train10 and Post12, Fig. 4, a, b).

This study indicated that 20-minute M1 a-tDCS (1 &2 mA) and M1 c-tDCS (1 & 2 mA) intervention was tolerated well with minimal adverse or side effects by all participants. The participants of all groups did not report any burning sensation or pain during or after stimulation. However, the majority of participants reported having itching during stimulation, which indicated a common side effect of the intervention.

***Integrity of blinding***

When the participants were asked to guess the nature of the tDCS conditions (active and sham), responses related to the active and sham interventions were included:

All active tDCS groups: active (n=16), sham (n=0), unsure (n=0); and sham group: active (n=16), sham (n=0), unsure (n=0). The differences in the active and sham guesses among the groups were not significant (x2=0.00, d.f.=1, p=0.97).

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**Discussion:**

The findings of the current study indicated that applying a-tDCS (1 and 2 mA) and also 2 mA c-tDCS improved online motor learning compared to the 1 mA c-tDCS and sham tDCS groups, with more significant effects of a-tDCS (1 and 2 mA). Moreover, the lasting effects of motor learning are also shown in a-tDCS (1&2 mA) and 2 mA c-tDCS groups. Additionally, the results indicated that 1 mA c-tDCS can disturb both online and offline motor learning.

**The effects of 1 or 2 mA M1 a-tDCS compared to sham tDCS on motor learning**

In the present study, we hypothesized that the groups receiving a-tDCS at intensities of 1 and 2 mA would show significant improvements in motor learning compared to the group receiving sham tDCS. The results of our study not only supported this hypothesis, but also revealed significant effects on both online and offline motor learning when compared to the sham group. These findings are consistent with previous studies conducted by Stagg et al. (2009, 2011), which investigated the efficacy of 1 mA M1 a-tDCS in motor learning and demonstrated a facilitative impact on online motor learning (Stagg *et al.*, 2009; Stagg *et al.*, 2011). Furthermore, evidence suggests that a-tDCS can enhance motor learning by facilitating offline consolidation effects (Reis *et al.*, 2009). Additionally, Ciechanski et al. (2017) found that healthy children exhibited increased motor learning and performance following 1 mA M1 a-tDCS compared to sham tDCS, with lasting effects observed up to 6 weeks after stimulation (Ciechanski & Kirton, 2017). Ambrus et al. also found that anodal stimulation increased offline learning (Ambrus *et al.*, 2012). According to the study by Greeley et al. (2020), the application of 2 mA M1 a-tDCS showed a faster reduction in RT compared to the sham group (Greeley *et al.*, 2020). Moreover, these individuals showed faster relearning after one year (Greeley *et al.*, 2020). It seems that 2 mA a-tDCS has broader and longer-lasting effects on corticospinal excitability, making it a more potent modulator of neuronal activity (Batsikadze *et al.*, 2013; Strube *et al.*, 2016). Overall, these studies collectively support our findings that a-tDCS at specific intensities can improve both online and offline motor learning outcomes compared to sham stimulation.

**The effects of 1 or 2 mA M1 c-tDCS compared to sham tDCS on motor learning**

According to the second hypothesis of the current study, c-tDCS with an intensity of 1 mA led to an inhibitory effect on online and offline motor learning. The findings of the current study supported this hypothesis. Similarly, Stagg et al. (2011) demonstrated that applying 1 mA c-tDCS decreased motor learning (Stagg *et al.*, 2011). Additionally, Pellicciari et al. (2013) showed that c-tDCS with an intensity of 1 mA led to a decrease in excitability and a reduced cortical response (Pellicciari *et al.*, 2013). On the other hand, Ciechanski et al. (2017) found that applying c-tDCS with a current intensity of 1 mA on the left M1 of right-handed children improved left-hand motor learning (Ciechanski & Kirton, 2017). It seems that the duration of c-tDCS application, the number of treatment sessions and the specific population under study may be the reasons of this controversy in the finding of the studies (Stagg *et al.*, 2011; Pellicciari *et al.*, 2013; Ciechanski & Kirton, 2017). However, the results of the current study indicate that c-tDCS with 1 mA current intensity not only doesn’t lead to enhance online and offline motor learning, but also induces inhibitory effects on online and offline motor learning.

We also hypothesized that applying M1 c-tDCS with a current intensity of 2 mA deteriorate online and offline motor learning. The findings of the current study did not support this hypothesis and indicated that 2 mA c-tDCS improved the motor learning with reducing RT during online learning and decreasing the ER during offline learning. These results are consistent with the findings of the study conducted by Greeley et al. (2020). In line with the study by Greeley et al. (2020), the group receiving 2 mA M1 c-tDCS demonstrated a faster reduction in RT compared to the sham group (Greeley *et al.*, 2020). Additionally, the findings of the study by Ciechanski et al. (2017) indicated that applying M1 c-tDCS with a current intensity of 2 mA area enhances motor learning in children compared to the sham group (Ciechanski & Kirton, 2017).

**Comparing effects of 1 mA M1 a-tDCS and c-tDCS**

It was hypothesized in the current study that 1 mA M1 a-tDCS compared to 1 mA M1 c-tDCS had more effects on online and offline motor learning. The findings of the current study were consisting with this hypothesis and even indicated disturbing effects of 1 mA c-tDCS as compared to 1 mA a-tDCS on motor learning. In this regard, there is evidence that applying M1 a-tDCS leads to an increase in excitability of the region through modulation of NMDA receptors, GABA receptors, BDNF, and calcium-dependent mechanisms (Liebetanz *et al.*, 2002; Nitsche *et al.*, 2003; Stagg *et al.*, 2009). It appears that anodal stimulation leads to a reduction in the activation threshold and ultimately increases the opening of voltage-dependent ion channels (Pellicciari *et al.*, 2013). By depolarizing the postsynaptic membrane and increasing the presynaptic firing frequency, it strengthens synaptic connections and improves learning (Pellicciari *et al.*, 2013). On the other hand, cathodal stimulation at 1 mA leads to decreased neuronal excitability due to an increase in the activation threshold (Pellicciari *et al.*, 2013). Additionally, reducing presynaptic activity and hyperpolarization in the postsynaptic membrane weaken synaptic connections (Pellicciari *et al.*, 2013). The results of the current study also indicated that M1 1 mA a-tDCs as compared to M1 1 mA c-tDCS induces more online and offline motor learning.

**Comparing effects of 2 mA M1 a-tDCS and c-tDCS**

In this study, it was also hypothesized that compared to 2 mA M1 c-tDCS, 2 mA M1 a-tDCS had more efficacy on motor learning. The findings of the current study confirmed this hypothesis and indicated although 2 mA M1 c-tDCS induced online and offline learning, more significant effects of 2 mA M1 a-tDCS as compared to 2 mA M1 c-tDCS were observed during online motor learning. However, lasting motor learning effects of 2 mA M1 c-tDCS were similar to 2 mA M1 a-tDCS. In the study conducted by Shilo et al. (2019), a comparison between M1 a-tDCS and c-tDCS with a current intensity of 2 mA during an SRTT task was performed (Shilo & Lavidor, 2019). According to the results of this study, during the initial stage of the SRTT task, execution speed was faster with anodal stimulation, while during the later stages, execution speed was faster with cathodal stimulation (Shilo & Lavidor, 2019). It appears that M1 c-tDCS with 2 mA current intensity leads to an increase in the intensity of motor-evoked potentials (MEP) and then inducing lasting motor learning (Batsikadze *et al.*, 2013). Accordingly, it seems that 2 mA M1 c-tDCS has a similar effect to 2 mA M1 a-tDCS, resulting in increased motor learning. In this regards, Bogaard et al. (2019) indicted that M1 c-tDCS with higher current intensity than 1 mA has a similar effect to a-tDCS, increasing the excitability of the targeted area (Bogaard *et al.*, 2019). Furthermore, Shilo et al. (2019) found that applying 2 mA M1 c-tDCS decreased the neural excitability for the first 13 minutes from 20 minute stimulation session, while the neural excitability increased and ultimately motor learning improved in final 7 minute of intervention (Shilo & Lavidor, 2019). In a study by Greeley et al. (2020), it was observed that applying both 2 mA M1 c-tDCS and a-tDCS improved long-term offline motor learning in individuals (Greeley *et al.*, 2020). This study suggests that c-tDCS and a-tDCS do not always have opposite effects and can have similar mechanisms under certain conditions (Greeley *et al.*, 2020). In a study conducted by Hsu et al., c- and a-tDCS with an intensity of 4 mA compared with the sham stimulation on motor learning. It was shown that a-tDCS compared to c-tDCS and sham stimulation improved significantly motor learning (Hsu *et al.*, 2023). Leow et al. compared the effects of the synergism of levodopa and a-tDCS with different intensities of 1,2 and 4 mA on motor learning and indicated that the application of levodopa concurrent with 4 mA a-tDCS causes a decrease, while concurrent with 1 and 2 mA a-tDCS causes an increase in motor learning compared to sham stimulation (Leow *et al.*, 2023). The current study also indicated the lasting effects of both M1 a-tDCS (1& 2 mA) and c-tDCS (2 mA) on motor learning in healthy young participants.

It seems that 2 mA c-tDCS generates excitatory after-effects by modulating the excitability of the motor cortex (Batsikadze *et al.*, 2013). This effect is achieved by inducing hyperpolarization in neurons (Batsikadze *et al.*, 2013). Additionally, the dopaminergic system plays a vital role in modulating the enduring effects of c-tDCS (Nitsche *et al.*, 2006). Research has highlighted the significance of dopamine in prolonging the excitability-diminishing effects of c-tDCS for up to 24 hours after stimulation, suggesting that tDCS may hold therapeutic potential for conditions characterized by increased cortical excitability (Nitsche *et al.*, 2006). These findings emphasize the complex relationship between neurotransmitter systems and the neuroplasticity induced by transcranial direct current stimulation (tDCS) (Nitsche *et al.*, 2006).

One of the limitations of the current study was that only the young healthy adults participated in this study, which limit the generalizability of these findings to this group. Conducting future studies to assess the efficacy of different intensities of a-tDCS and c-tDCS on motor learning in the other aging individuals is suggested. Another limitation in the current study was the absence of evaluation of neural activity both during and after interventions, which hinders the ability to elucidate the mechanisms underlying cortical activity changes following tDCS intervention. It is recommended to conduct a study that evaluates the excitability of the M1 by employing TMS-EEG techniques after the application of a-tDCS and c-tDCS at varying intensities.

**Conclusion:**

Employing M1 a-tDCS at various intensities (1 & 2 mA) and M1 c-tDCS (2 mA) may prove beneficial in augmenting motor learning among young healthy adults. It appears that opting M1 a-tDCS or c-tDCS with an intensity of 2 mA and also M1 a-tDCS with an intensity of 1 mA are suitable strategies for the consolidation phase of motor learning. Additionally, the findings highlight a detrimental impact associated with M1 c-tDCS (1 mA). As a result, it is not recommended for therapists to utilize M1 c-tDCS with intensity of 1 mA for introducing motor learning in young healthy adults.

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**Statement of Ethics:**

This study was approved by the Human Ethics Committee at the Neuromuscular Rehabilitation Research Center of Semnan University of Medical Sciences (IR.SEMUMS.REC.1401.284), according to the declaration of Helsinki. The study, as a clinical trial study, was also registered in the Iranian Registry of Clinical Trials (www.irct.ir; IRCT20221023056277N2).

The informed consent form was completed by all participants before enrolment stage. In addition, written informed consents were obtained from participants.

**Conflict of interest:**

Sheida Mousavi, Amin Mottahedi, Cyrus Taghizadeh Delkhosh, Fatemeh Ehsani and Shapour Jaberzadeh declare that they have no conflict of interest. The authors had not any support and financial involvement (e.g. employment, consultancies, honoraria, stock ownership and options, expert testimony, grants or patents received or pending, royalties) in the previous three years. Also, the authors declare that they had not any financial relationships (personal, political, or professional) that may potentially influence the writing of the manuscript.

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Cyrus Taghizadeh Delkhosh: Data collection, Manuscript editing

Fatemeh Ehsani: Protocol/project development, Management Data analysis, Manuscript writing

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**Data Availability Statement**

Data of this article is available, if it is required.

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**Figure captions:**

**Fig. 1** Flow diagram of participant’s eligibility assessment

**Fig. 2** A flow diagram of the serial response time testing conditions during pre-test, main test, and post-test

**Fig. 3** Reaction Time (RT, Mean ± SEM ) of blocks during serial response time test (SRTT) training in (**A)** 1mA M1 a-tDCS group, (**B)** 2mA M1 a-tDCS group, (**C)** 1mA M1 c-tDCS group, (**D)** 2mA M1 c-tDCS group, (**E)** sham M1 tDCS group, Error Rate(ER, Mean ± SEM) of blocks during SRTT training in (**F)** 1mA M1 a-tDCS group, (**G)** 2mA M1 a-tDCS group, (**H)** 1mA M1 c-tDCS group, (**I)** 2mA M1 c-tDCS group, (**J)** sham M1 tDCS group; \* indicates significant differences within group

**Fig. 4 A** The post-hoc analysis comparison of online learning (changes in RT between blocks 3 and 10): a-tDCS (1mA) Vs. c-tDCS (1 mA) group (p=0.001), a-tDCS (1 mA) Vs. sham tDCS group (p=0.01), c-tDCS (1 mA) Vs. a-tDCS (2 mA) group (p=0.01);, offline learning (changes between blocks 2 and 12): a-tDCS (1 mA) with c-tDCS (1 mA) group (p=0.01), a-tDCS (1 mA) with sham tDCS group (p=0.02) and consolidation effect of learning (changes between blocks 10 and 12) among groups; **B** the comparison of online learning (changes in ER): a-tDCS (1 mA) Vs. sham tDCS group (p=0.037); offline learning: a-tDCS (1 mA) Vs. c-tDCS (1 mA) group (p=0.001), c-tDCS (1 mA) Vs. c-tDCS (2 mA) group (p=0.03), a-tDCS (2 mA) Vs. c-tDCS (1 mA) group (p=0.001), a-tDCS (1 mA) Vs. sham tDCS (p=0.002) group, a-tDCS (2 mA) Vs. sham tDCS group (p=0.028) and consolidation effect of learning among groups (Mean differences ± SEM)