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Timing of high-definition transcranial direct current stimulation to the nondominant primary motor cortex fails to modulate cortical hemodynamic activity and improve motor sequence learning

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Abstract

Background The relative timing of transcranial direct current stimulation (tDCS) and motor practice holds potential importance in modulating cortical activity and facilitating behavioral performance.

Method A single-blind, randomized, cross-over experiment was conducted. Twenty healthy participants engaged in a sequential finger-tapping task with their left hand. High-definition anodal tDCS (1 mA, 20 min) was administered over the right primary motor cortex (M1) either during (concurrent-tDCS) or before the motor practice (prior-tDCS). A sham tDCS condition was also employed. The three tDCS conditions were separated by one-week intervals. Cortical hemodynamic activity in the prefrontal cortex (PFC), supplementary motor area (SMA), and M1 measured by functional near-infrared spectroscopy, as well as motor performance assessed by number of correct sequences were examined before (T1), immediately after (T2), and 24 h after the practice (T3). The data was subjected to a two-way repeated measures analysis of variance.

Results No significant interaction or main effect of condition were found on motor performance. Regarding cortical hemodynamic activity, none of the regions of interest or channels exhibited a significant interaction effect or main effect of condition. No significant correlation between cortical activity and motor performance was found.

Conclusion Our results cannot support the timing effect of single-session anodal tDCS on facilitating brain activity or improving motor performance. These results contribute to the growing body of evidence challenging the efficacy of a single session of exogenous stimulation as an adjunct to motor practice for promoting motor acquisition. Further research should explore alternative tDCS parameters, multiple sessions and various age groups.

Keywords Transcranial direct current stimulation, Motor learning, Timing-dependent effect, Functional near-infrared spectroscopy

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Background

Transcranial direct current stimulation (tDCS) is a non-invasive brain stimulation technique that could modulate cortical excitability and facilitate neuroplasticity by delivering weak direct currents over the scalp. Over the past decades, tDCS targeting the motor cortex, combined with motor practice, has been developed to treat poststroke motor impairment [1, 2]. One crucial consideration when integrating tDCS with motor practice is the timing of the stimulation. Research indicated that synchronizing tDCS with a motor task specifically enhanced learning, as opposed to interleaved stimulation and movements [3]. This concurrent stimulation was assumed to act by promoting coincident mechanisms of plasticity in the circuits of the brain that are active during the movement. Our previous research also observed that bilateral tDCS (with anodal electrode of the lesioned primary motor cortex, M1) applied concurrently with mirror therapy in patients with chronic stroke significantly improved upper extremity motor performance compared to those receiving prior or sham tDCS [4]. However, evidence remains inconsistent across healthy and clinical populations [5–11], with one study observing significantly improved kinematic outcomes only in participants receiving prior stimulation [10] and several reporting no timing effect [6, 8, 11].

Neurophysiological and neuroimaging techniques allow for assessing the resultant cortical activity evoked by the timing of tDCS, enhancing our understanding of how timing modulates neuroplasticity within the cerebral cortexes, which is essential for tDCS treatment optimization. Previous studies primarily used electromyography to measure the corticospinal excitability [12–14]. However, electromyography studies failed to detect significant differences in brain activity triggered by stimulation timing, possibly because they missed the instantaneous neural changes from the interaction between tDCS after-effect and movements.

Functional near-infrared spectroscopy (fNIRS) emerges as a promising measurement. It can be conducted with electrical stimulation without interference and allows participants to move relatively freely while measuring immediate cortical response to neurostimulation [15] or motor tasks [16]. The potential mechanisms and the state-of-the-art utilization of fNIRS in conjunction with tDCS can be found in a recent review [17]. fNIRS assessment on modulation of tDCS on the cortical motor area started in 2013 [18]. To the best of our knowledge, only one study has explored the timing effect of tDCS on a motor task and hemoglobin responses using fNIRS; the authors observed a greater increased oxygenated hemoglobin concentration change (ΔHbO) when tDCS conducted concurrently with finger opposition [11]. However, this study did not set up a practice process, which is an essential element for motor learning [19].

Here, we sought to investigate whether the timing-dependent effect of tDCS found in our previous work [4] could occur in age-matched healthy individuals and examine the accompanied hemoglobin response via fNIRS by stimulating the non-dominant hemisphere. We targeted the non-dominant hand because of its reduced dexterity resulting from asymmetric use compared with the dominant hand [20], which could mimic the differences between the paretic and non-paretic sides in patients with stroke. Moreover, anodal tDCS is particularly likely to facilitate motor function in the non-dominant rather than the dominant hand [21]. Thus, we expected great room for improvement by stimulating the non-dominant hemisphere. Based on our previous observation in stroke patients, we hypothesized that concurrent tDCS and motor training (concurrent-tDCS) would elicit better motor learning and greater cortical hemodynamic activity than prior (prior-tDCS) or sham tDCS (sham-tDCS).

Methods

Study design

A single blind randomized cross-over experiment was conducted, wherein participants received three tDCS conditions (i.e., concurrent-tDCS, prior-tDCS, and sham-tDCS) at a random order. The random sequence was generated by Microsoft Excel and was counterbalanced across participants. Conditions were separated by one week apart and were performed during the same time in a day.

Participants

Twenty right-hand dominant, healthy adults (mean age ± SD: 60.2 ± 9.47; 12 males and eight females) voluntarily participated in this study from August 2022 to November 2022. The sample size was determined based on a previous study [11]. Using the effect size of 0.636, calculated from the partial-eta squared values of 0.288 [22], an alpha of 0.05 and a power of 0.95, a minimum sample size of 9 was determined using the G*Power software (ANOVA: Repeated measures, within factors, http://www.gpower.hhu.de/). To ensure adequate power and the ability to have reliable estimates and replicable findings, we aimed to include a sample size of 20. Our study was performed in accordance with the principles of the Declaration of Helsinki and was approved by the human ethics committee of Shanghai Yangzhi Rehabilitation Hospital (Shanghai Sunshine Rehabilitation Center) before implementation (reference No.SBKT-2021-092).

Participants were assessed for eligibility based on the following inclusion criteria: (1) passed the safety screening for tDCS; (2) had no prior history of neurological or

psychiatric disorders or upper extremity injuries; and (3) had normal or corrected vision. All participants gave written informed consent after being informed of the study content and potential adverse effects.

Experimental setup

A high-definition tDCS (HD-tDCS) stimulator (Soterix Medical Inc) was used. Five sintered Ag/AgCl electrodes were attached to plastic holders, filled with conductive paste, and embedded in a fNIRS cap. We administered anodal stimulation targeting the right Brodmann area 4 (primary motor cortex) by positioning an active anode electrode on C4 surrounded by four return electrodes on FC2, FC6, CP2, and CP6.

A continuous wave fNIRS system (NIRSport, NIRx Medical Technologies LLC., USA) was used to evaluate ΔHbO in the regions of interest (ROIs). The fNIRS system utilizes two wavelengths (~760 and 850 nm) at a sampling rate of 8.72 Hz. A total of 14 channels primarily covered the bilateral prefrontal cortex (PFC, left: Channel 1; right: Channel 2), SMA (left: Channel 3, 5; right: Channel 4, 6), and M1(left: Channel 11 to 14; right: Channel 7 to 10) regions, with 16 optode probes (8 sources × 8 detectors) placed in the custom, 10 – 5 based arrangement cap to accommodate tDCS electrodes (see Fig. 1a). The cap was designed with standardized surface positions for the human head, ensuring approximately 3.0 cm spacing between any two neighboring positions.

Protocol

The experiment was conducted in a quiet room. After instrumentation setup, the participant was seated comfortably on an armchair with both shoulders flexed at 45° and forearm pronated on a table.

Participants got initially familiarized to perform a sequential finger-tapping task using their nondominant hand (left for all participants in this study) (see Fig. 1b) [23]. This task is considered a valid substitute for investigating the mechanisms underlining real-life motor skills acquisition and is easily implemented in restrictive conditions like neuroimaging environments, making it a classic experiential paradigm to measure motor sequential

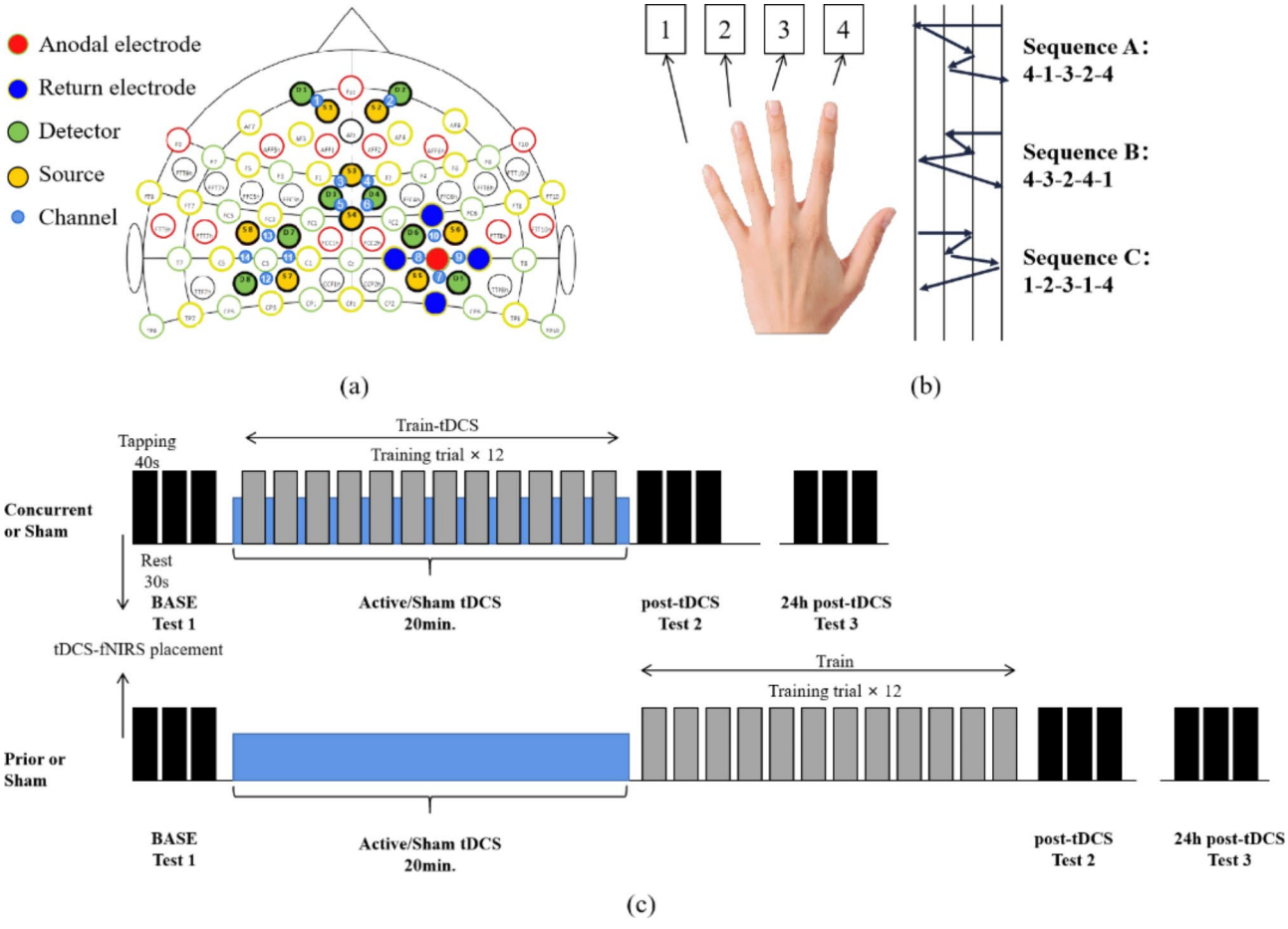

Fig. 1 Experimental design. (**a**) fNIRS – tDCS montage. (**b**) The sequential finger-tapping task. Participants were required to press four numeric keys in a rapid and accurate manner with their index [4], middle [3], ring [2] and fourth finger [1]. (**c**) Experimental design. For sham-tDCS condition, participants randomly underwent concurrent or prior paradigms

learning [24]. Participants were required to press four numeric keys in a rapid and accurate manner, repeating the three five-element sequence: week 1: sequence A (4-1-3-2-4), week 2: sequence B (4-2-3-1-4), and week 3: sequence C (1-3-2-4-1) 25). The sequences were placed across the center of the computer screen, against a black background, and were constantly displayed throughout the task to eliminate any working memory component. The task program was created and presented using the E-prime software (version 3, Psychology Software Tools, Inc.) Each sequence task was organized in an alternative block design, with each block consisting of a 40-s task period followed by a 30-s rest and repeated three times. The familiarization phase concluded once participants achieved 80% accuracy, after which they were instructed to take a 5-min break.

Participants were then assigned to receive one of the three tDCS conditions. Each condition is composed of four phases as depicted in Fig. 1(c): [1] Time 1 (T1): pre-stimulation sequential finger-tapping task; [2] 20-min active or sham tDCS: in the concurrent-tDCS condition, 12 blocks of task practice conducted concurrently with the stimulation, commencing 3 min after the initiation of stimulation. In the prior-tDCS condition, task practice was carried out 5 min after the completion of the stimulation; [3] Time 2 (T2): post-stimulation sequential finger-tapping task [4]. Time 3 (T3): 24-h post-stimulation sequential finger-tapping task.

To maintain participant blinding, the stimulator monitor was concealed from view. Additionally, a 30-second ramp-up and ramp-down period was employed to further ensure participant blinding.

Preprocessing of fNIRS data

The Homer2 toolbox in Matlab (version R2020a, The MathWorks Inc., USA) was used to preprocess fNIRS data [26]. The fNIRS signal quality was tested by checking the cardiac power of each channel using spectrum analysis [27]. Channels with poor signal quality were excluded from further preprocessing. Then raw data were pruned using the *enPruneChannels function*. After converting the raw intensity data into optical density changes, motion artifacts were examined and corrected sequentially by *hmrMotionArtifactByChannel* (tMotion = 0.5, tMask = 2.0, StdevThresh = 20 and AmpThresh = 0.2) and *hmrMotionCorrectSpline*. Finally, a temporal low-pass filter (< 0.1 Hz) and a high-pass filter (> 0.01 Hz) were employed to attenuate the effect of physiological noises and drifts, respectively. An integral between − 5 and 45 s relative to the onset of blocks was computed for block averaging visualization. A baseline correction was applied using a -5 to 0 s time window. As approximately 5 s is needed for HbO to increase from pre-stimulation to a stable concentration change, a time interval between 5 and 35 s after the block onset was used for calculating the mean ΔHbO induced by the three conditions [28].

tDCS-related adverse effects and blinding assessment

The potential side effects experienced were evaluated using the tDCS adverse effects questionnaire [29], with severity measured by the Visual Analog Scale (VAS). We adopted the end-of-study guess approach to determine the blinding efficacy. Participants were asked to guess if they had received active or sham stimulation after each condition.

Statistical analysis

Normal Q-Q plots and box plots were used to check outliers. Data more than three standard deviations from the mean were flagged as outliers [30, 31]. Whether outliers were excluded in subsequent analyses was determined after thoroughly reviewing the data collection process and assessing the impact of these deviant data using Cook's distance. Cook's distance exceeding 1 were considered to indicate highly influential outliers [32]. The average of multiple channels serving as one ROI on ΔHbO was calculated for ROI-level statistical analysis. For ΔHbO, we also analyzed each individual channel in case of a nonsignificant result in the ROI-level comparison. All statistics were calculated using SPSS (version 23.0; SPSSInc.IL, USA, Chicago). The cortical hemodynamic activity and motor performance (number of correct sequences) were subjected to a two-way repeated measures analysis of variance (ANOVA), with two main effects (Condition: concurrent-tDCS, prior-tDCS, and sham-tDCS; Time: T1, T2, and T3) and one interaction effect (condition × time). The significant level was set at $p < 0.05$ for the ROI level analysis and adjusted to $p < 0.05$ divided by the number of channels for each ROI at the channel-level analysis. Mauchly's test of sphericity was employed to assess the homogeneity of variance. If the sphericity assumption was violated, we reported the result from Greenhouse-Geisser epsilon adjustment. The multiple comparisons problem in post-hoc analysis was corrected by the Bonferroni method. The relationship between ΔHbO and motor performance (number of sequences and its normalized index) was examined for each channel in different conditions by calculating partial correlation coefficients and controlling for age and sex as confounding variables.

A Fisher's exact test was conducted to compare the number of participants guessing active, sham or expressing uncertainty across three conditions, assessing the success of blinding. An additional two-way ANOVA was conducted for sham-tDCS to evaluate whether the expectation of real stimulation would be related to improved motor performance and significant changes in cortical hemodynamic activity.

Results

Motor performance

The two-way ANOVA revealed no significant interaction effect ($F = 1.39$, $p = 0.25$) or main effect on condition ($F = 1.00$, $p = 0.38$), but a significant time effect was observed ($F = 50.81$, $p < 0.0001$) (see Supplementary Table S1). Refer to Fig. 2 for trajectories of the number of sequences completed in each stimulation condition.

Cortical hemodynamic activity

Channel 13 and 14 from Participant 17, channel 6 from Participant 19 in the prior-tDCS condition, channels 2 and 12 from Participant 11 in the concurrent-tDCS condition, and channel 3 from Participant 18 in the sham-tDCS condition at follow-up were excluded from further analysis. Missing data for channels 3, 6, and 12 to 14 were imputed by the data from surrounding channels within the same ROI. The expectation-maximization algorithm was applied to obtain maximum likelihood estimates for the missing value in channel 2 [33]. Two outliers from Participant 19, identified in the left SMA and M1 at T1 under the concurrent-tDCS condition, were retained due to their good signal quality and minimal influence, as shown by the Cook's distance.

Table 1 presents the results of a two-way repeated measures ANOVA in terms of the main effects of time and condition and their interaction effect on ΔHbO in the PFC, SMA, and M1. None of the ROIs exhibited a significant interaction effect or main effect of condition (all $ps > 0.05$). A significant time effect was observed in the left PFC ($F = 4.36$, $p = 0.02$) and bilateral M1 (left: $F = 5.63$, $p = 0.007$, right: $F = 7.46$, $p = 0.002$) (Fig. 3). An interaction effect was found in Channel 7 in the stimulated M1, whereas the p-value did not pass the correction for multiple channels ($p = 0.031$).

The correlation between cortical hemodynamic activity and motor task performance

No significant correlation was found between ΔHbO and the number of sequences at T2 and T3. Furthermore, our investigation into the relationship between changes in HbO levels at T2 and T3, relative to T1, and motor performance did not yield any significant correlations.

tDCS-related adverse effects, blinding succuss and placebo effects

The most commonly reported adverse effects were tingling (VAS ≤ 2, 65%), pain (VAS ≤ 2, 41.7%) and itching (VAS ≤ 2, 30%). Table 2 listed the distribution of responses for the end-of-study guess and the results of Fisher's exact test. The responses of "I don't know", "active guesses" and "sham guesses" were evenly distributed across the three conditions, suggesting successful blinding. The two-way ANOVA examining the presence of a placebo effect revealed no significant differences in motor learning and cortical hemodynamic activity among participants who guessed active in the sham-tDCS condition

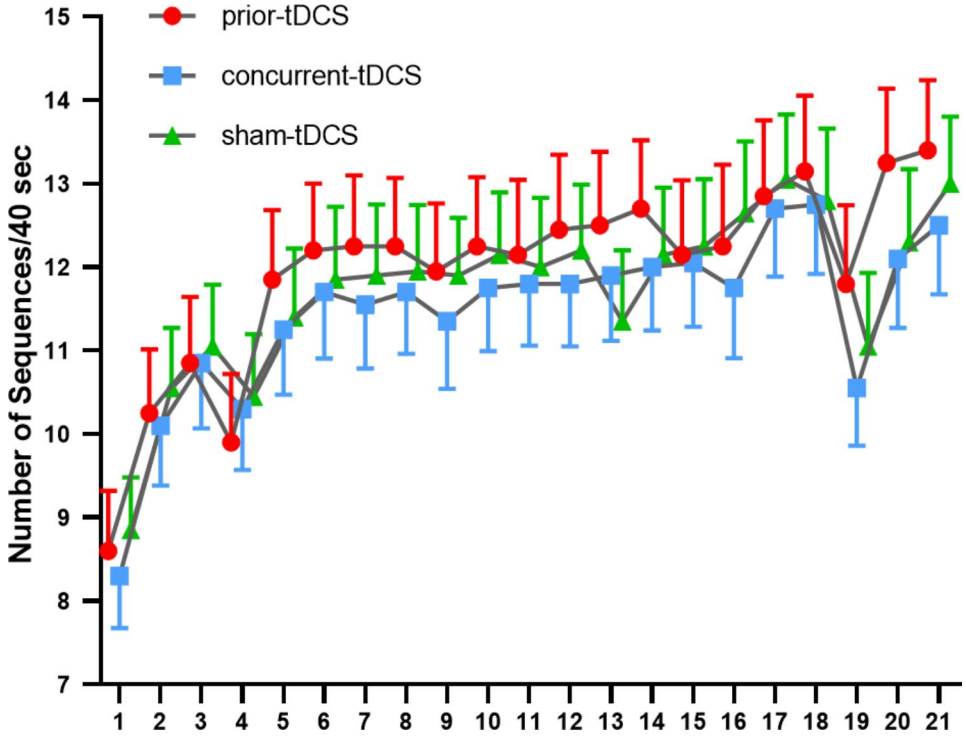

Fig. 2 Trajectories of the number of sequences completed in the prior-tDCS, concurrent-tDCS, and sham-tDCS conditions

Table 1 Two-way ANOVA analysis on ΔHbO in the PFC, SMA and M1

Cortical region			Prior-tDCS			Concurrent-tDCS			Sham-tDCS			Main effect				Interaction effect	
			T1	T2	T3	T1	T2	T3	T1	T2	T3	Condition		Time			
												F value	*p* value	F value	*p* value	F value	*p* value
PFC	L	ROI	0.51 ± 0.54	0.56 ± 0.63	0.66 ± 0.56	0.76 ± 0.49	0.45 ± 0.42	0.59 ± 0.59	0.70 ± 0.64	0.37 ± 0.49	0.79 ± 0.85	0.17	0.85	4.36	0.02*	1.87	0.15
	R	ROI	0.51 ± 0.55	0.54 ± 0.65	0.78 ± 0.81	0.68 ± 0.57	0.43 ± 0.49	0.56 ± 0.68	0.45 ± 0.57	0.46 ± 0.63	0.51 ± 0.75	1.04	0.36	1.16	0.32	1.14	0.35
SMA	L	ROI	0.21 ± 0.19	0.17 ± 0.21	0.18 ± 0.28	0.26 ± 0.22	0.18 ± 0.18	0.18 ± 0.23	0.29 ± 0.31	0.24 ± 0.30	0.28 ± 0.16	1.63	0.21	2.31	0.11	0.24	0.92
		CH3	0.20 ± 0.22	0.12 ± 0.20	0.18 ± 0.27	0.28 ± 0.26	0.21 ± 0.21	0.17 ± 0.25	0.32 ± 0.40	0.27 ± 0.39	0.29 ± 0.25	2.24	0.12	2.08	0.14	0.37	0.70
		CH5	0.21 ± 0.21	0.22 ± 0.30	0.19 ± 0.31	0.24 ± 0.23	0.15 ± 0.20	0.19 ± 0.24	0.26 ± 0.25	0.20 ± 0.27	0.27 ± 0.16	0.62	0.54	1.14	0.33	0.52	0.72
	R	ROI	0.20 ± 0.28	0.19 ± 0.29	0.20 ± 0.37	0.31 ± 0.26	0.27 ± 0.26	0.26 ± 0.20	0.26 ± 0.26	0.22 ± 0.33	0.22 ± 0.31	1.27	0.30	0.78	0.47	0.07	0.99
		CH4	0.19 ± 0.27	0.18 ± 0.30	0.24 ± 0.32	0.34 ± 0.25	0.31 ± 0.30	0.25 ± 0.24	0.29 ± 0.28	0.25 ± 0.31	0.14 ± 0.52	1.53	0.23	0.91	0.41	0.79	0.54
		CH6	0.21 ± 0.32	0.20 ± 0.31	0.16 ± 0.48	0.28 ± 0.28	0.24 ± 0.24	0.28 ± 0.20	0.23 ± 0.25	0.18 ± 0.38	0.30 ± 0.25	0.79	0.46	1.07	0.34	0.78	0.54
M1	L	ROI	0.38 ± 0.37	0.25 ± 0.46	0.39 ± 0.40	0.40 ± 0.46	0.32 ± 0.37	0.31 ± 0.44	0.46 ± 0.32	0.14 ± 0.49	0.52 ± 0.55	0.15	0.86	6.59	0.003*	1.83	0.13
		CH11	0.36 ± 0.56	0.15 ± 0.62	0.35 ± 0.43	0.40 ± 0.53	0.38 ± 0.39	0.29 ± 0.55	0.50 ± 0.37	0.24 ± 0.50	0.64 ± 0.95	1.94	0.16	3.75	0.03*	1.22	0.31
		CH12	0.25 ± 0.51	0.20 ± 0.65	0.22 ± 0.53	0.27 ± 0.45	0.26 ± 0.36	0.20 ± 0.49	0.35 ± 0.47	-0.06 ± 0.66	0.41 ± 0.56	0.03	0.97	3.99	0.03*	2.2	0.08
		CH13	0.40 ± 0.39	0.34 ± 0.54	0.42 ± 0.40	0.40 ± 0.48	0.23 ± 0.43	0.38 ± 0.42	0.41 ± 0.34	0.14 ± 0.80	0.55 ± 0.61	0.17	0.85	4.23	0.02*	1.07	0.38
		CH14	0.51 ± 0.50	0.27 ± 0.55	0.58 ± 0.55	0.53 ± 0.55	0.40 ± 0.53	0.32 ± 0.57	0.60 ± 0.46	0.22 ± 0.57	0.48 ± 0.71	0.71	0.89	4.80	0.01*	1.40	0.24
	R	ROI	0.57 ± 0.40	0.39 ± 0.40	0.62 ± 0.49	0.60 ± 0.48	0.56 ± 0.41	0.62 ± 0.54	0.58 ± 0.33	0.33 ± 0.52	0.71 ± 0.48	0.66	0.52	7.46	0.002*	1.18	0.33
		CH7	0.44 ± 0.41	0.31 ± 0.60	0.54 ± 0.51	0.45 ± 0.68	0.70 ± 0.65	0.43 ± 0.55	0.48 ± 0.48	0.33 ± 0.54	0.72 ± 0.70	0.73	0.49	1.86	0.17	2.82	0.031*
		CH8	0.57 ± 0.48	0.47 ± 0.58	0.59 ± 0.56	0.62 ± 0.57	0.63 ± 0.54	0.66 ± 0.57	0.63 ± 0.44	0.33 ± 0.72	0.63 ± 0.53	0.92	0.41	1.90	0.16	0.87	0.49
		CH9	0.59 ± 0.48	0.34 ± 0.44	0.69 ± 0.63	0.62 ± 0.59	0.58 ± 0.59	0.71 ± 0.63	0.69 ± 0.55	0.43 ± 0.67	0.74 ± 0.54	0.83	0.44	6.48	0.004*	0.63	0.64
		CH10	0.70 ± 0.50	0.43 ± 0.67	0.65 ± 0.51	0.72 ± 0.42	0.34 ± 0.84	0.67 ± 0.62	0.53 ± 0.78	0.24 ± 1.24	0.73 ± 0.54	0.30	0.74	5.16	0.01	0.39	0.82

Data was expressed as mean ± SD. L = left, R = right, PFC = prefrontal cortex, SMA = supplementary motor area, M1 = primary motor cortex

* $p < 0.05$

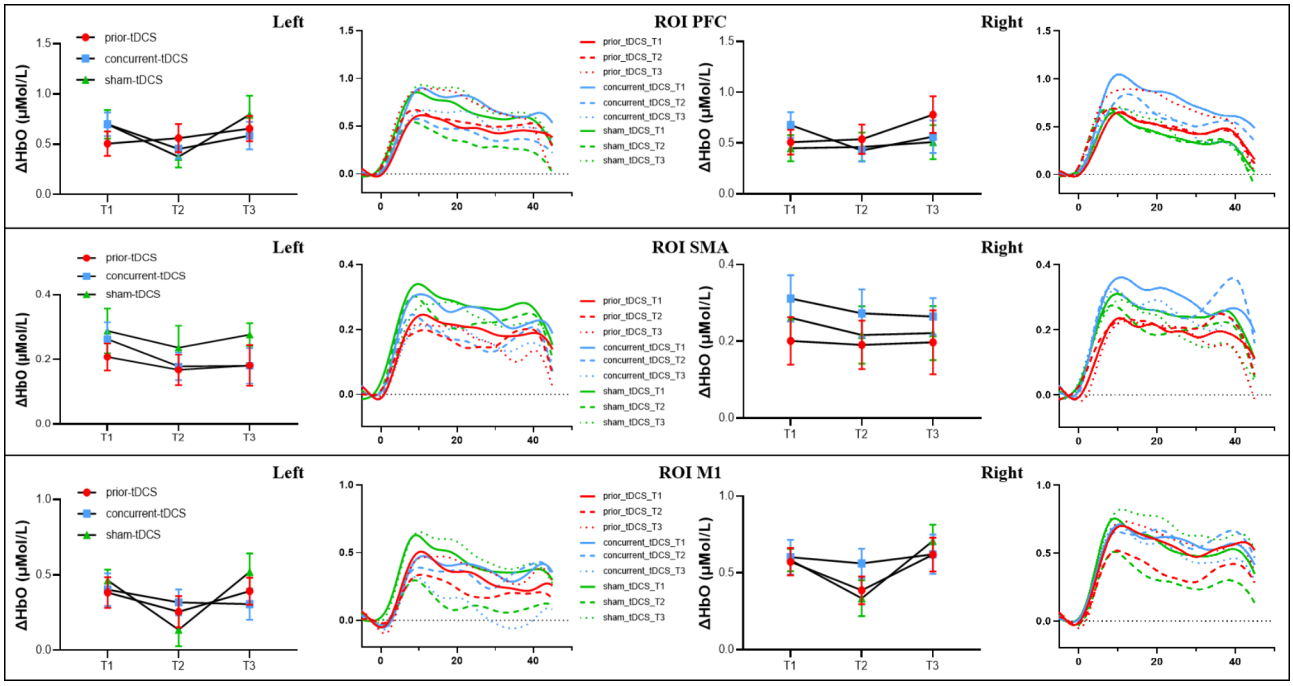

Fig. 3 Evolution and post hoc analysis of ΔHbO in the prior-tDCS, concurrent-tDCS, and sham-tDCS conditions

Table 2 The distribution of responses for the end-of-study guess and the results of the Fisher's exact test

Condition	Prior-tDCS	Concurrent-tDCS	Sham-tDCS	Fisher's exact test statistic	*p*
I don't know	6/20 (30%)	7/20 (35%)	8/20 (40%)	0.49	0.94
Active Guesses	12/20 (60%)	11/20 (55%)	10/20 (50%)	0.45	0.95
Sham Guesses	2/20 (10%)	2/20 (10%)	2/20 (10%)	0.22	1

compared to those who guessed sham or were uncertain (all $ps > 0.05$; see Supplementary Table S2).

Discussion

This study measured and compared the motor task-evoked cortical hemodynamic activity in old healthy individuals before and after a single session of HD-tDCS under three conditions: concurrent-, prior- and sham-tDCS. Contrary to our hypothesis and previous findings, no significant timing-dependent effect of a single session of tDCS was found, as indicated by changes in cortical hemoglobin levels and the number of sequences completed.

The effect of single session tDCS on motor performance and cortical hemodynamic activity

In healthy subjects, the study by Nitsche et al. (2003) [34] was the first to show the beneficial effects of a single session of anodal tDCS over the M1 for improving motor sequences. However, subsequent investigations have revealed inconsistent results [35]. Particularly, some meta-analyses indicated that one session of M1 anodal tDCS seems not to be enough to induce significant behavioral changes compared to sham stimulation [36]. Our results, in line with a growing body of literature, failed to replicate the previously reported effects of tDCS on motor sequences, implicating the high variability in response to tDCS effect.

Research has indicated that tDCS could produce sustained cortical excitability increases in healthy populations for up to 30 [37] to 90 min [38] following the stimulation. However, large variability exists [39, 40], with approximately half of individuals having minimal or no response to 2 mA tDCS either during or 30 min after stimulation [40]. This unstable effect of anodal tDCS on motor cortical excitability was further confirmed in a large double-blind placebo-controlled trial [41]. Compelling fMRI studies also have reinforced the variability in activation alterations after tDCS stimulation [42]. Considering the good spatial and temporal correlation between cortical hemodynamic changes measured by fNIRS and the BOLD response from fMRI [16], these fMRI findings may offer insights into our inability to detect significant changes in cortical hemoglobin levels between active and sham tDCS after stimulation.

Additional considerations regarding the present negative results

To thoroughly discuss the potential reasons underlining our non-significant results, we summarized the population characteristics, tDCS parameters and main findings of previous studies that used similar paradigms in the Supplementary Table S3. On the behavioral level, four out of ten studies found that the timing of anodal tDCS altered the trajectory of motor acquisition, and consistently reported that concurrent-tDCS significantly improved motor performance compared to prior-tDCS [4, 5, 7, 12]. However, there is no direct evidence supporting this timing effect on cortical activity changes.

Since positive observations are derived from studies employing various motor tasks and tDCS parameters in different populations, we cannot determine the pattern most likely to produce a timing-dependent effect. However, we have considered the following points for future research.

First, our negative results might be attributed to age-related alterations in motor sequential learning linked to changes in the neural circuits, particularly in the corticostratal network [43–45]. We recruited participants with an average age of 60.2 to match our previous work [4] and the typical age of a first-ever stroke in the Chinese population [46]. Although older adults retain the capability to learn motor skills, motor sequence learning is selectively impaired [47], as the cortical plasticity is weakened, especially in the motor cortex, reducing the efficiency of brain motor functions [48–50]. Multisession anodal tDCS has been reported to enhance motor cortex plasticity and motor learning in the aging population [51], whereas a recent study combining three consecutive daily sessions of motor sequence practice with tDCS over right M1 did not facilitate learning in healthy older adults [52]. Conversely, a meta-analysis indicated that a single session of tDCS, whether applied before or during motor task practices, significantly improves motor performance in older subjects [53]. Thus, the benefits of tDCS, regardless of the number of stimulation sessions, in combination with motor tasks in healthy older people remain unclear.

The selection of 1 mA for stimulation intensity was informed by our previous work, where a significant timing-dependent effect was observed in stroke patients. Earlier evidence showing such effects also chose lower intensities, with three using 1 mA [4, 5, 12] and one 1.5 mA [7]. While it is generally accepted that the electric field induced by tDCS will increase linearly with current intensity [54], this linear dose-response relationship has not been confirmed in neurophysiological and behavioral responses [55–57]. Another piece of evidence even pointed out that an intensity ≥ 1 mA might shift long-term potentiation- into long-term depression-like plasticity [58]. However, given that the dose-response relationship for tDCS is complicated and most investigated in younger subjects, we cannot determine if low-intensity stimulation accounts for the lack of positive findings.

Strengths and limitations

We employed a HD-tDCS stimulator in the present study, as modelling studies demonstrated that conventional large pad tDCS produces a wide-spread electric field, and the largest current density might fall outside the target electrode [59, 60]. HD-tDCS montages, using smaller electrodes, were developed to increase the density and focality of current. The arrays of 4×1 ring configuration on the targeted cortex reduce the diffusion of tDCS-induced electric fields [61], thereby generating neuroplasticity more focally than conventional tDCS [62, 63]. Furthermore, we specifically assessed motor performance and cortical hemodynamic activity after the completion of motor practice, which differs from previous studies that examined the effect during concurrent motor task and stimulation [11], where the real difference between the concurrent-tDCS and prior-tDCS effect might be contaminated.

Nevertheless, several methodological issues warrant discussion. First, we did not arrange for another researcher to manage the sham mode to blind the interventionist. However, we designed a standardized operating procedure and ensured consistent instructional language across all conditions. Our data was collected using Aurora fNIRS (NIRSport Acquisition software) and E-prime, minimizing the bias related to the interventionist's awareness of the stimulation modalities. Nevertheless, future studies are suggested to be carefully designed as double or even triple-blinded. Secondly, we only targeted the nondominant M1. Stimulating novel targets like the dorsolateral prefrontal cortex, which is involved in the initial-phase motor skill acquisition when cognitive control processes are required [54, 64], might provide alternative opportunities to detect positive results. The last issue is that we did not have short separation channels or an extra detector to create short channels. It has been reported that stimulation electrodes can cause an approximate 1 °C increase [65], leading to variations in skin blood flow and HbO signals [66]. The presence of unrelated extracerebral hemodynamic activity may dramatically impact the outcomes [67].

Conclusions

The current findings suggest no significant timing-dependent effect of single-session anodal tDCS on cortical hemodynamic activity and motor sequential learning. However, considering the methodological issues we mentioned in the limitations (e.g., the lack of interventionist blindness and short separation channels), it is premature

to conclude that tDCS offers no benefit for motor skill acquisition or there is no timing effect of tDCS in neurophysiological and behavior responses. Further research is warranted to explore alternative tDCS parameters, multiple sessions and various age groups to reach a more definitive conclusion.

Abbreviations

ΔHbO	Changes in concentrations of oxygenated hemoglobin
ANOVA	Analysis of variance
BOLD	Blood-oxygenation-level-dependent
fMRI	Functional magnetic resonance imaging
fNIRS	Functional near-infrared spectroscopy
HD-tDCS	High-definition transcranial direct current stimulation
M1	Primary motor cortex
PFC	Prefrontal cortex
ROIs	Regions of interest
SMA	Supplementary motor area
tDCS	Transcranial direct current stimulation
VAS	Visual analog scale

Supplementary Information

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Supplementary Material 1

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Not applicable.

Author contributions
ZB and MJ were responsible for conceptualizing and designing the study. XL and WX were involved in the recruitment and screening of participants. XX and ZZ were involved in the data collection process. MJ conducted the data analysis and was the primary author responsible for drafting the manuscript. MJ, ZB and WX were responsible for revising the manuscript. All authors read and approved the final manuscript.

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Data availability
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The study was approved by the human ethics committee of Shanghai Yangzhi Rehabilitation Hospital (Shanghai Sunshine Rehabilitation Center) before implementation (reference no. SBKT-2021-092).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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