# Dose-response of tDCS effects on motor learning and cortical excitability: a preregistered study

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# Abstract

Neuromodulatory effects of transcranial direct current stimulation (tDCS) on the primary motor cortex (M1) have been reported in terms of changes in corticospinal excitability using motor evoked potentials (MEPs), as well as behavioral effects during motor skill learning. While both effects are thought to be mediated by synaptic plasticity, empirical evidence of a common neural substrate is lacking. Complicating matters, the effects are relatively small, leading to mixed results, or even reversing effects when applying currents up to 2mA. In this ongoing preregistered study we aim to determine the dose-response of tDCS on cortical excitability and motor skill learning at higher current intensities in humans. In a double-blind, sham-controlled, counterbalanced design, 120 healthy subjects are assigned to one of three groups (N=40 each), receiving either 4mA, 6mA, or 0mA tDCS. tDCS is applied concurrently targeting M1, while the subject performs a 12-minute long explicit sequence learning task using their left hand. Using transcranial magnetic stimulation (TMS), motor evoked potentials (MEPs) are measured on the left first dorsal interosseus (FDI) immediately before and after the task. TMS-evoked potentials (TEPs) are recorded simultaneously with MEPs using electroencephalography (EEG) as a measure of cortico-cortical excitability. The trial is powered to test for a monotonic or non-monotonic dose-response relationship between tDCS intensity and motor skill performance as well as change in MEP amplitude. Correlation between physiological and behavioral effects would provide support for the notion that these stimulation effects share a common neural substrate. More importantly, this trial aims to establish whether tDCS can have robust effects on motor skill learning.


# Introduction

Transcranial direct current stimulation (tDCS) has been widely studied as an intervention to modulate cortical brain activity. *In vitro* animal studies have shown that electric fields induced by electrical stimulation can polarize the somatic membrane of neurons[1], which can in turn increase or decrease neuronal firing rates[2,3]. Changes in cortical excitability have been observed *in vivo* in human experiments that target the primary motor cortex (M1).[4] These experiments measured motor evoked potentials (MEPs) in a hand muscle elicited by transcranial magnetic stimulation (TMS) over the motor cortex. This cortico-spinal excitability is increased following “anodal” tDCS (with inward current flow at the region of interest) and a decrease following “cathodal” tDCS (with outward current)[4]. tDCS effects on the motor system can also be assessed behaviorally, as seen in some experiments that found that anodal stimulation applied over M1 enhanced motor skill learning[5–7]. It is widely believed that the two

phenomena have a common physiological substrate, such as a modulation of synaptic efficacy involved in motor skill learning [8–18]. While there is ample evidence that various interventions with electric and magnetic stimulation can modulate MEPs[19–22], we are not aware of any human behavioral studies showing an effect of motor skill learning on MEPs. Also, the studies on the effects of tDCS on motor skill learning have given mixed results[23–25], and there is skepticism around reproducibility of MEP effects of tDCS[26,27]. Some null results of tDCS on MEP may be explained as reversal of effects at higher intensities[28] leading to a “no man’s land” at intermediate intensities[29]. Our primary hypothesis is that of a monotonic effect of tDCS, but we remain open to the possibility of a reversal of the effects on MEP or motor skill learning. If the dose response of MEP reverses with increasing tDCS intensity, but is monotonic for skill learning (or vise versa), it would be more difficult to reconcile this with a common physiological substrate.

We previously theorized that consistency of *in vivo* human tDCS effects may be improved by increasing tDCS intensity, based on our earlier *in vitro* findings of a monotonic dose-response of field strength in synaptic plasticity[30,31]. However, *in vitro* current densities are much higher than those applied in human studies, so dose effects may not translate[32,33]. Animal models and *in vitro* experiments typically apply 5 V/m or higher, compared to the relatively low estimated fields in humans of less than 1 V/m at 2 mA[34]. Although effects have been demonstrated *in vitro* under electric fields as small as 0.2 V/m with alternating currents[2], the dose-response of direct currents below 2.5 V/m has not been investigated. Furthermore, since current intensities in most of the human studies literature were limited at 2 mA, characteristics of tDCS effects at higher levels are largely unknown[33]. Within the scope of this study we focus on the dose response of anodal tDCS, as effects of cathodal stimulation are asymmetrical[28,30,35–37]. There is evidence of a positive effect of anodal tDCS intensity on motor learning performance at intensities up to 1.5 mA[38]. This follows a similar monotonic dose-response at up to 2 mA in cerebral blood flow (CBF)[39,40], but contradicts other studies that have consistently found a nonlinear relationship with MEP amplitude at up to 2 mA[41–46]. Notably, all but one of these works cited ([46]) did not employ neuronavigation, which is generally thought to improve TMS accuracy[47] and, more importantly, targeting stability[48]. It is less clear whether a similar nonlinear effect continues above 2 mA for behavioral learning effects. Agboada et al. found that 1, 2, and 3 mA all increased MEP amplitudes, but without significant differences across the intensities[37]. On the other hand, Shinde et al. demonstrated a monotonic dose-response of tDCS across 0.1, 2, and 4 mA in both CBF and behavioral outcomes in the region of interest under the anode. We also found a positive behavioral effect on motor skill learning at 4 mA[49], but those results remain to be replicated. In total, there is conflicting literature on the dose-response of tDCS on both motor skill learning as well as MEPs. To address this, we will test the dose-response of tDCS up to 6mA, which is made possible by the use of HD electrodes to mitigate sensation by spreading out current intensity on the scalp[49].

The conflicting dose-response relationships call into question, additionally, the hypothesis that behavioral and MEP effects have the same physiological substrate. Indeed, changes in M1 activity during and following motor learning as observed with neuroimaging[18,50] are not reflected consistently in MEP amplitude changes. While simple repetitive movements can affect M1

excitability[18,50–53], studies have found that sequence learning tasks do not[54,55]. MEP changes resulting from use-dependent plasticity during motor practice is evidently linked to GABA inhibition[56,57]. Although GABA concentration can be reduced by tDCS[58], motor learning with tDCS does not appear to yield a consistent correlation between MEP amplitude and GABA synaptic activity or motor skill acquisition[23,59]. Time may be yet another factor, as weeks of skill training have been shown to increase excitability[17], in contrast with strength training[60]. Even in the absence of skill learning, tDCS effects on MEP amplitudes are evidently state-dependent, varied by M1 activation with a simple motor task[61,62]. We will measure both performance and excitability changes, and the presence or absence of a correlation between them may help address some of these uncertainties around what neural pathways are modulated by tDCS.

We are motivated by the causal model of mechanistic effects presented in Fig. 1. Here the effect of learning and electric fields generated by tDCS interact to affect both motor performance and MEP amplitude. The factor mediating this interaction (white box) is commonly thought to be synaptic plasticity, but the current experiment does not measure this directly, so all we are assuming is that there is a common physiological substrate. Based on this model we hypothesize a monotonic relationship between performance on a motor skill learning task and tDCS intensity (H1), and a monotonic relationship between MEP change and tDCS intensity (H2). We hypothesize that when controlled for tDCS intensity, motor learning task performance is positively correlated with change in MEP amplitude (H3). As an exploratory objective, we will also observe changes in cortical excitability as measured through TMS-evoked potentials (TEPs) using electroencephalography (EEG), as opposed to corticospinal excitability measured in MEPs[63–66].

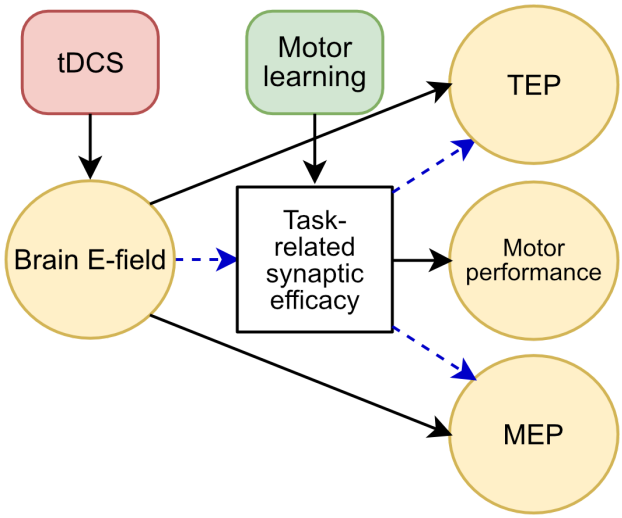


**Figure 1. A proposed model of tDCS action and interaction with motor skill learning in human studies.** We experimentally control tDCS intensity (red) to determine its effect on experimental outcome variables (yellow). Solid lines indicate established causal effects. For instance, motor skill learning (green) is thought to causally affect motor performance via changes in task-related synaptic efficacy in M1. Similarly, transcranial currents produce electric fields in the brain that are thought to modulate MEP and TEP magnitudes. Dashed lines indicate causal mechanisms hypothesized here that are less well established. For instance, while there is clear *in vitro* evidence that electric fields can modulate synaptic efficacy, it is not clear that this is the mechanism by which tDCS affect performance outcomes in motor

skill learning. We previously postulated an interaction between behavioral training and electric fields to affect synaptic efficacy (i.e. both are required)[30]. It is often assumed that electric fields affect MEPs via a modulation of synaptic efficacy, but evidence for this is conflicting, as discussed in the main text.

The use of TEPs as an indicator of plasticity and excitability changes following tDCS is a relatively recent development. Like MEPs, TEP amplitudes have been found to increase after anodal tDCS and even correlate with MEP amplitudes[61,67]. More recently, Mosayebi-Samani et al. found a nonlinear dose response of TEPs to cathodal tDCS over M1 at up to 2.1 mA[68]. Whereas 1.4 mA tDCS resulted in an increase in early TEP amplitudes but no effect on MEP amplitudes, 0.7 mA and 2.1 mA tDCS resulted in decreased TEP amplitudes and MEP amplitudes, with significant positive correlations. We will collect similar measurements of cortical excitability and determine whether TEPs can capture the synaptic changes occurring during motor learning with tDCS. Due to a lack of standardization of TMS-EEG techniques and analysis[66], we have not committed to a preplanned analysis pipeline for TEP data. Based on the results from the few existing studies with tDCS, we hypothesize a monotonic relationship between tDCS intensity and TEP amplitude. We also expect positive correlations between TEP amplitudes and performance as well as MEP amplitudes.

Finally, this study may serve as a partial replication of our previous study at 4 mA where we also tested whether tDCS effects outlasted the period of stimulation and whether they were specific to the stimulated hemisphere and task. The experimental design therefore closely matches the previous study[49].

# Methods

## Experimental Design

Subjects will be algorithmically assigned to one of three groups, each corresponding to a different stimulation condition. At the beginning of the experiment, each subject will complete a typing test to assess baseline typing speed that will serve to control for dexterous skill. Group assignment will be selected at random while minimizing variation in typing speed across groups. The study will be double blinded by delegating different tasks between two researchers. One researcher will be responsible for monitoring the group assignments, as well as setting the stimulation intensity on the stimulator and operating the stimulator. The stimulator settings will be obscured from the second researcher, who will prepare subjects for tDCS and explain the behavioral task to the subject. The second researcher, who is blinded to the tDCS condition, will also conduct the TMS and MEP data collection procedures before and after tDCS, while the first researcher may assist. Code for data preprocessing and analysis will be written and tested without knowledge of individual subjects' stimulation condition, until the final reporting of the outcome variables listed in the Analysis Plan below.

## Subjects

This study will be conducted on healthy right-handed adults, consisting primarily of students recruited on the campuses of the City University of New York. Informed written consent will be obtained from all participants for being included in the study, under approval by the City University of New York Institutional Review Board. Prospective participants will be screened and excluded if there are any contraindications to TMS and tDCS. These include a history of seizures, fainting, or head trauma; pregnancy; chronic headaches, nausea, and drowsiness; metal implants in the head; wounds or chronic skin disorders on the scalp; thick and tightly braided hairstyles; weaves and wigs; and any headgear that cannot be removed. For the purpose of this study, other exclusion criteria included left-handedness; neurological or psychiatric disorders; taking nervous system medication; severe visual impairment; chronic abuse of psychoactive substances; and disability of the left upper extremity. Subjects with prior experience of tDCS will be excluded from the study to facilitate blinding. Subjects will be asked to not consume alcohol or other psychoactive substances (except caffeine) on the day of the experiment. Individuals with experience in playing musical instruments involving sequential finger movements will also be excluded from the study, unless they have stopped playing for a number of years exceeding the years of experience.

## Procedures

Procedures will follow the timeline outlined in Fig. 2c. Up to 2 experimental sessions will be conducted per day, starting either at 9:00 AM or 1:00 PM.

### Typing Test and Group Assignments

The subject will first be asked to complete a baseline task to determine their baseline dexterous skill. We elected to use a simple typing test, as keyboard typing has been associated with better manual dexterity, visuomotor coordination, and motor speed[69–71]. This task is broadly recognizable as most adults are already experienced to some extent, making it easy to administer with minimal instruction. It is also sufficiently different from the main task, so as to minimize interference with the actual sequence learning task. Importantly, we found in a pilot study that there is a considerable positive correlation (r = 0.58) between baseline typing speed and baseline sequence learning performance (see below: “Predicting motor learning ability”). Subjects will be asked to correctly type a series of 63 common English words as quickly as possible using both hands. The words will appear in a sequence of 22 characters on the screen (including spaces), with the text scrolling along leftward as the subject correctly types out characters. A stationary cursor will highlight the leftmost character to be typed next. The task will continue until all characters have been pressed correctly. There will be no time limit on this task, but we estimate that it can typically be completed in approximately one minute. The results from this baseline task will be evaluated using a script in MATLAB (MathWorks, Natick, MA) that will also manage group assignments. Baseline task performance will be measured as words per minute (WPM). Initially, the first three subjects will be sequentially assigned to the three groups. Subsequent subjects will then be sorted by the grouping that results in the smallest summed variance across groups, as described by Sella et al.[72] This form of “covariate-adaptive” method

of minimization, originally conceived by Taves[73] and Pocock and Simon[74], can help limit imbalances in randomized controlled trials[72,75].

## MEP Measurement

MEPs will be measured immediately before and after tDCS (Fig. 2). The subject will be seated on a chair with an arm rest and asked to relax their arm. Electromyography (EMG) recordings will be collected using an eego mini EMG amplifier (ANT Neuro, Hengelo, Netherlands) from the left first dorsal interosseous (FDI) muscle through foam electrodes and triggered by single-pulse TMS using a MEGA-TMS system (Soterix Medical, Woodbridge, NJ) with a standard figure-of-eight coil (100 mm diameter). TMS pulses will be delivered to the M1 region representing the FDI, which will be located using a visor2 neuronavigation system (ANT Neuro, Hengelo, Netherlands). Starting from the C4 electrode location on the 10-10 system, we will search using 1 cm steps in the coronal and sagittal planes for the FDI “hotspot” that yields the highest MEP amplitude at a given output level. The increments will be lowered to less than 5 mm as we approach the “hotspot”, continuing until the MEP amplitude appears to stop increasing. The coil will be oriented at a 45 degree angle toward the midline. Resting motor threshold (RMT) will be determined as the minimal output level necessary to produce a MEP in at least six out of ten pulses. 60 pulses will be delivered at 120% RMT, with at least 5 seconds between pulses. After measurement of the left FDI MEPs, the same procedures will be applied to the right FDI, stimulating over the left M1, near the C3 electrode location. The same TMS output level will be used before and after tDCS and training. Neuronavigation will ensure precise placement of the coil across multiple trials and sessions such that the same cortical location can be stimulated consistently[47,48]. For this purpose, individual anatomical magnetic resonance images (MRIs) are not required, as instead we will rely on “hotspotting” to locate the FDI representation in M1. Where MEP protocols can range from 20 to 100 TMS pulses[4,67,77], we opted for 60 pulses as suggested for reliability in TEP measurements[66]. The total number of TMS trials falls within historical safety guidelines[78] and the amount used for motor mapping[79].

## TEP Measurement

EEG will be recorded simultaneously with TMS-MEP measurements using a TMS-compatible amplifier (BrainVision, Gilching, Germany) and TMS-compatible Ag/AgCl sintered “C” electrodes (BrainVision, Gilching, Germany) attached to a custom EEG cap (EasyCap, Wörthsee, Germany) with cutouts for HD1 Electrode Holders (Soterix Medical, Woodbridge, NJ). A custom 32-channel electrode layout will be used, with electrode positions redistributed around the HD1 cutouts (Fig. 2b). Because we are interested in TEPs at M1 (approximately C3 and C4), the adjacent positions will be more densely populated. Electrode wires will be wired away from C3 and C4 and oriented such that there is a 90 degree angle with the TMS coil in order to limit TMS artifacts in the signal[80]. The EEG layout will be symmetrical across the midline such that the recordings will not be biased toward either hemisphere. Data will be sampled at 5 kHz and epoched around triggers sent by the TMS.

## tDCS

tDCS will be delivered using the same layout used in our previous study[49] (Fig. 2b). Using a high-definition (HD) tDCS system (M×N-9, Soterix Medical, Woodbridge, NJ), current will be spread out across four electrode pairs to limit skin sensation, and electrodes will be placed in a montage that optimizes electric field intensity at the M1 representation of the left hand fingers. Ag/AgCl sintered ring HD electrodes (Soterix Medical, Woooodbridge, NJ) will be attached to the head through the custom EEG cap with low-profile HD-tDCS electrode holders[81] to allow close proximity of the TMS coil to the scalp. Conductive gel (Signagel, Parker Laboratories, Fairfield, NJ) will be applied on the hair under the electrodes in the same manner as EEG preparation. Anodal stimulation (inward current on the targeted cortical structure) will be delivered in the posterior-anterior direction with anodes placed over the right parietal lobe at P4, CP4, CP2, and P2, and cathodes placed over the center-right frontal lobe at F4, F2, AF4, and Fz. A reference electrode will be placed over CP3. The current through each electrode pair will be either 1 mA for a total of 4 mA; 1.5 mA for 6 mA total; or 0 mA. When the stimulator is activated, current intensity will ramp up gradually to full intensity over 30 seconds, and similarly ramp down over 30 seconds when the stimulator is turned off. The tDCS device constantly monitors impedance during stimulation to ensure that every channel is below 10 kΩ, thus reducing the likelihood of an adverse reaction on the scalp. Once the current reaches full intensity, we will ask the subject whether the sensation is acceptable, and if so, whether they would like to proceed to the task. Throughout the experiment the participant is reminded that they can ask to end the stimulation at any point, for both TMS and tDCS.

The 0 mA group will receive 30-second ramped sham stimulation up to 1 mA total at the beginning, immediately followed by a 30-second ramp down. This low intensity will be used because tDCS at 4 mA (and above) is very noticeable and cannot be reasonably sham controlled[49], whereas the difference is more subtle at 1 mA. After stimulation has ended, the subject will also be asked whether they think they received verum or sham stimulation (or don't know). We do not expect to achieve comparable levels of placebo effects from skin sensation alone, since participants under the sham condition will experience significantly lower levels. Nonetheless, as discussed below in the Pilot Data section, we do not expect a correlation between sensation and motor performance. Sham stimulation at 1 mA may not be effective in a within-subject design[82–85], but in this case the subjects will not have multiple stimulation conditions to compare across. Therefore, we expect all subjects to perceive some level of stimulation.

## Motor Sequence Learning Task

The subject will perform the same task used in our previous study, following the same procedures and sequences[49] (Figure 2a,c). They will be seated facing a computer monitor, with their left hand fingers resting on a four-key response pad labeled with the digits "1", "2", "3", and "4" from left to right. The monitor will display a MATLAB-based graphical user interface (GUI). To avoid any possible experimenter bias, all instructions for the task will be delivered through the GUI. During each trial of the task, the GUI will show a five-element sequence consisting of the digits "1", "2", "3", and "4", e.g. "4-1-3-2-4"[9,86]. The subject will be instructed to press the

matching keys sequentially in the order shown on the GUI, as quickly and as accurately as possible. Each time any key is pressed, the GUI will display an asterisk above the most recent digit in the sequence, regardless of correctness. The initial task will be performed with the left (non-dominant) hand using a first sequence (L:S1) and paired with tDCS. It will be repeated 60 minutes later without stimulation to test for lasting effects. To test for any lasting effect on the unstimulated hemisphere, the task will then be repeated without stimulation on the right hand on a new sequence (R:S2). We will also test a new sequence on the left hand (L:S3) to see whether tDCS can boost subsequent learning on the stimulated hemisphere.

## Sensation Rating

After the stimulation is turned off, the subject will be asked to rate skin sensation levels of tDCS perceived at three different time points of stimulation: at the beginning of stimulation, at the middle of stimulation, and after the stimulation is turned off. Ratings will be on a Wong-Baker visual analog scale[87] from 0 to 10, with 10 being the most severe. One or more qualities of the sensation will also be rated from the options: “No sensation”, “Tingling”, “Pricking/Stinging”, “Itching”, “Burning”, “Other”. Subjects will also be asked whether they believed they received stimulation: “Do you think you received stimulation? (Yes, No, Not sure)”

The IRB protocol for TMS and tDCS includes an adverse event reporting form which asks the participant to report any of the following: headache, neck pain, scalp pain, tingling, burning sensation, skin redness, sleepiness, trouble concentrating, acute mood changes, and nausea/lightheadedness/dizziness. We will report these ratings as secondary safety outcomes in this study.

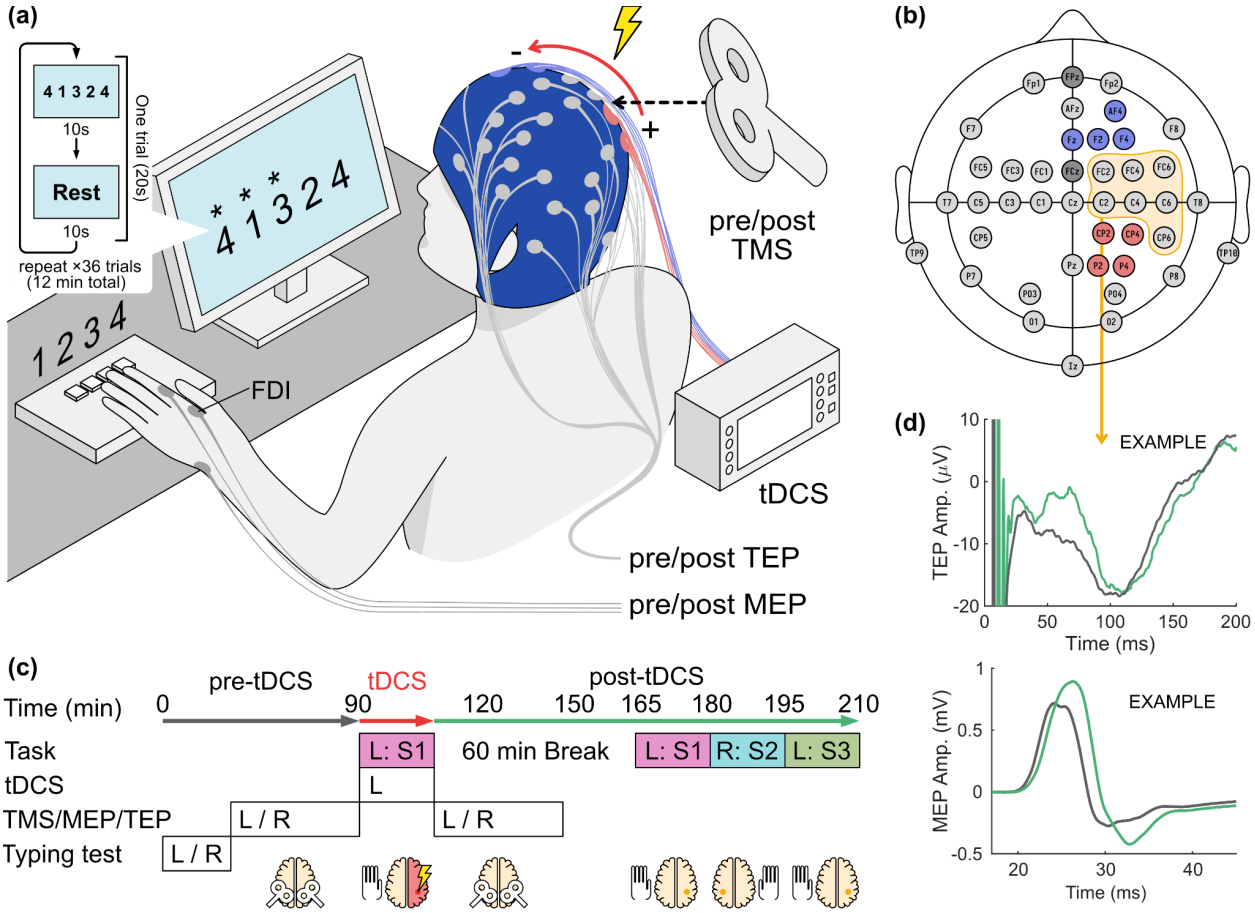


**Figure 2. Experimental setup and timeline. (a)** The subject will receive "anodal" tDCS over the right M1 during 12 minutes of motor sequence learning (Task) performed using the left (non-dominant) hand. A monitor will display a five-element sequence for the subject to replicate by pressing four buttons with their left hand fingers. Each finger will press one key labeled with a corresponding number. TMS will be applied pre- and post-tDCS to measure MEPs and TEPs. **(b)** A custom electrode cap will be used to both deliver tDCS and record EEG. Electrical stimulation will be spread out across four parietal "anodes" (red) and four frontal "cathodes" (blue) in the same locations used previously 40. 32 EEG channels (light gray) will be distributed symmetrically across the midline and around the tDCS electrode locations. **(c)** The subject will first complete a baseline typing task to determine group placement based on baseline typing speed. TMS-evoked MEPs will be measured from both the left and then the right FDI before and after the initial sequence learning and tDCS. At 60 minutes after the end of tDCS and initial task training, the subject will repeat the sequence learning task to test whether learning effects are lasting (R:S1), specific to hemisphere (R: S2), and specific to the trained sequence (L:S3). **(d)** Local TEPs will be sampled from the electrodes close to the hand area of M1, approximately at the C3 and C4 locations. Pre/post amplitudes will be compared, as shown in the example (gray/green respectively). Likewise, pre/post MEP amplitudes from the same TMS trials will be compared, as shown in the example.

## Data Exclusion

Only data from subjects who have completed all TMS, MEP, EEG, and tDCS procedures will be included. MEP and TEP analysis will exclude trials that are three quartiles away from the median (in their log of power). In our hands, stimulation artifacts in TEP can vary considerably

across subjects and electrodes. We will exclude electrodes that show stimulation artifacts lasting more than 25 ms. Outliers in task performance will also be excluded. We will exclude subjects who show signs of inattentiveness, i.e. overall accuracy less than 50% in keypresses, any consistent pauses longer than 3 seconds when keypresses are expected, or any trials where no response is recorded.

## Analysis Plan

**Table 1. Planned analyses.** See Pilot Data section for details.

| **Question** | **Hypothesis** | **Outcome Measures** | **Sampling Plan** | **Analysis Plan** | **Rationale for deciding the sensitivity of the test** | **Interpretation given different outcomes** | **Theory falsifiable by the outcomes** |
|---|---|---|---|---|---|---|---|
| Does dose of concurrent tDCS have an effect on motor sequence learning? | H1: Performance differs with tDCS dose. | Average number of correct sequences (NCS) with Left hand on Sequence S1 (L:S1) | N=40 per group yields >90% power | Linear model with intensity as a graded variable and typing speed as covariate. If no significant effect is found: see Fig. 3. | Effect size of Cohen's d=0.56 between 4mA to 0mA from previous data[49] was used as the basis for power analysis. | p > 0.05: see Fig. 3 for follow-up analyses.<br><br>p < 0.05: tDCS effect on behavior is monotonic with intensity. | tDCS modulates learning-related plasticity. |
| Does dose of concurrent tDCS have an effect on corticospinal excitability? | H2: Monotonic increase of MEP change with tDCS dose. | Post-Pre MEP amplitude ratio | Assuming $\eta^2$ = 0.12, power = 95% | Linear model with intensity as a graded variable. | Effect size assumed here is much more conservative than a previous study ($\eta^2$ = 0.91[67]) | p > 0.05: nonlinear or no effect on MEP<br><br>p < 0.05: monotonic effect on MEP | tDCS modulates cortical excitability. |
| Is motor sequence learning associated with changes in MEP? | H3: Performance correlates with MEP change. | NCS, post-pre MEP amplitude ratio | N=40 points per group can resolve a significant association with 90% power and r ≥ 0.29 | Linear mixed effect model with MEP as fixed effect and subject as random effect | Assuming a fixed N as computed above, we determined resolvable effect size. | p < 0.05 is consistent with a linear effect of tDCS on a common cause of MEP change and motor learning. | Effects of tDCS on MEP and motor skill are similar and share the same neural substrate. |

Data will be analyzed using MATLAB, following the plans detailed in Table 1 and Figure 3. Power analyses are based on our previous behavioral data and described in more detail in the Pilot Data section. The main performance outcome for each subject will be calculated exactly as done previously[49], by taking the average number of correct sequences (NCS) across all trials of the first iteration of the task, concurrent with stimulation. MEP amplitudes for each subject will be calculated by averaging the raw amplitudes across 60 EMG epochs triggered by the TMS pulses. The average will only include trials with a detectable biphasic MEP. Change in MEP amplitudes will be calculated by taking the ratio of the measurement post-tDCS over pre-tDCS.

These primary outcomes will test hypotheses H1 on performance and H2 on change in MEP, and H3 will test for a correlation between these two effects.

Because we have prior data on behavior, we will apply a more rigorous analysis pipeline for this outcome (Fig. 3). If a significant effect is found in the initial linear model with intensity as a graded variable (and typing speed as a covariate), intensity would be interpreted as a monotonic effect, since we do not expect to resolve a significant difference between 4 and 6 mA. In the case where no significant effect is found at the first stage, we would test a follow-up linear model with intensity as a categorical variable. A significant finding at this second stage would be followed by a Tukey HSD pairwise comparison between 4 and 6mA. A significant difference between the two groups would be interpreted as a "reversing" effect, whereas a lack of a difference would be interpreted as a "saturating" effect, with linearity ruled out. A non-significant finding at the second stage would be interpreted as a lack of effect from tDCS intensity. Post-hoc analyses in the second and third stages will be Bonferroni-corrected accordingly. In the case of a null finding resulting in a saturating dose response or no effect, we will use Bayes factor analysis to measure the evidence in support of the corresponding null hypothesis using an established MATLAB toolbox[89].

Exploratory analyses include a test for lasting effects and carryover effects as observed in our previous study[49]. There we did not find a significant performance difference after 1 hour nor between the different hands or sequences. We will test whether these effects replicate in the current study by repeating the same analyses of the number of correct sequences. MEP and TEP effects on the right hand will be exploratory. Although they may serve as a within-subject control, there may be behavioral carryover effects, and the high tDCS intensities may cause parts of the left hemisphere to be stimulated. All TEP outcomes will be exploratory. We are interested in whether there is an effect of tDCS dose on changes in TEP, and whether those effects are hemisphere specific. We would also like to observe any possible correlation between performance, MEP, and TEP effects. In general, we will look at post-pre local mean field power (LMFP) around the region of interest around C4 and C3, sampled from the electrodes in the vicinity (Fig. 2b), as well as global mean field power across all channels. As an a priori measure, we expect to see a significant across-group difference in post-pre LMFP ratio within the 25-60 ms period after the TMS pulse, as reported by Ahn and Frohlich[67]. EEG data will be processed using original scripts and the EEGLAB package for MATLAB[88]. All results will be evaluated at a significance level of $\alpha = 0.05$. If any significant dose effect is found, post-hoc analysis will consist of Tukey HSD to evaluate pairwise differences between individual groups. We will report descriptive statistics for samples organized by time of day (morning vs. afternoon), age, sex, and stimulation condition.

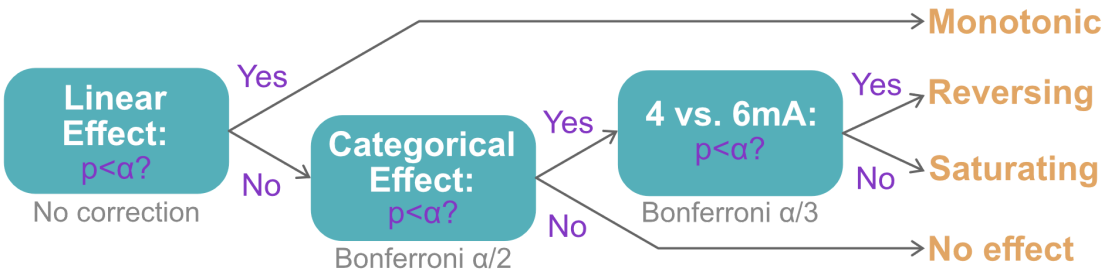


**Figure 3. Analysis pipeline for primary behavioral outcome.** An initial linear model at the first stage of analysis will use intensity as a graded variable and typing speed as a covariate. If no significant effect is found, a second stage analysis will use intensity as a categorical effect. If a significant effect is found, a third stage analysis will apply a Tukey HSD pairwise comparison between 4 and 6 mA. Second and third stage analyses will be Bonferroni-corrected accordingly, with α = 0.05. The interpretations of the dose effect given these possible outcomes are “monotonic”, “reversing”, “saturating”, and “no effect”.

# Pilot Data

## tDCS at 6 mA

Following our findings of a positive effect on skill learning performance at 4 mA (Fig. 4a)[49], we collected an additional group of N=32 that received 6 mA tDCS using the same protocol, under approval by the City University of New York Institutional Review Board. Current flow modeling using ROAST[90] (realistic volumetric approach to simulate transcranial electric stimulation) was conducted a priori in consideration of safety. Simulations were run on the same 10 anatomical MRIs we used in our previous study to formulate the electrode montage[49]. Applying the 1.5 mA per electrode pair under our previous configuration for a total of 6 mA, we found that the maximal electric field achieved on the surface of the brain was approximately 1.8 V/m, which corresponds to an estimated current density of 0.23-0.50 A/m$^2$ in the brain[91]. This is well below the threshold for tissue damage at approximately 50 A/m$^2$ for 30 minutes[92]; in other words, >100 mA would be necessary to induce hazardous levels of current density in the brain. While currents as low as 4 mA passed through a single electrode may cause adverse effects[93–95], here we limited stimulation to 1.5 mA per electrode. Additionally, the base area of the gel in the Soterix HD1 Holder is approximately 4.5 cm$^2$, through which a current of 1.5 mA would yield approximately 0.33 mA/cm$^2$ current density on the skin per electrode. Since this is below the upper tolerability limit of 0.5 mA/cm$^2$ commonly cited in iontophoresis literature[96], we expect this level of stimulation to be tolerable.

A one-way ANOVA on the behavioral outcomes showed a significant effect of current on performance (mean number of correct sequences per trial) during the initial training task ($F(3,136) = 3.76$, $p = 0.012$). We found in a post-hoc Tukey’s HSD test that the 6 mA group performance was not significantly different from those of the -4 and 0 mA groups ($p = 1.0$, $p = 0.96$, respectively), resulting in a non-monotonic relationship overall (Fig. 4a). Contrary to our hypothesis, 6 mA did not appear to confer any benefit over no stimulation. However, we suspect

that there may have been inhomogeneity across the different cohorts, since the data were collected over 1 year apart on a new cohort of subjects. Replication of the previous experiment and validation of these results is therefore an important goal of the planned experiment. 6 mA tDCS was well tolerated at a rating of around 4 on a visual analog scale from 0 to 10[87] (Fig. 4b), with no difference in sensation rating at the beginning of stimulation from -4 and +4 mA (one-way ANOVA; $F(2,100) = 1.59$, $p = 0.21$).

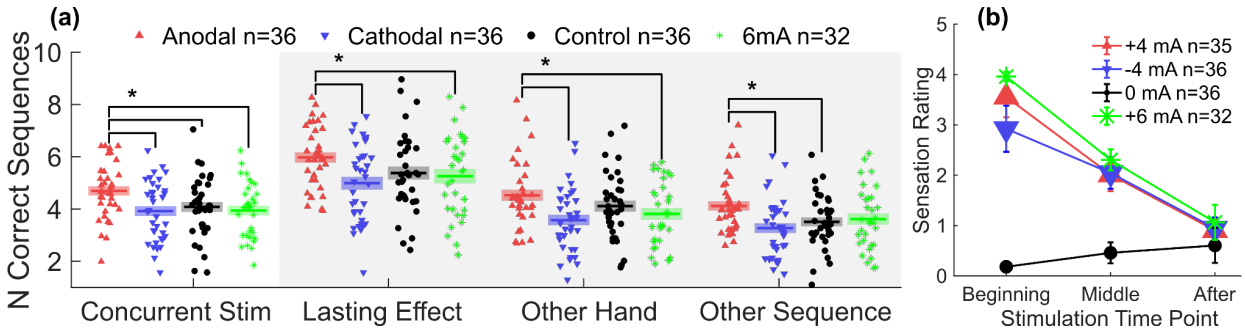


**Figure 4. Preliminary results combining published results with a 6 mA group that was collected later. (a)** Overall performance in the 6 mA group was not significantly different from those of the -4 and 0 mA groups. Each point represents performance by one subject averaged across all trials. Horizontal bars represent group average and shaded areas represent standard error of the mean (SEM). Brackets represent differences with $p < 0.05$. **(b)** Sensation ratings rated on a visual analog scale from 0 to 10, with 10 being the most severe. Average sensation ratings for 6 mA were slightly higher, but not significantly different from +4 and -4 mA ratings. Error bars represent SEM.

# Power analysis and predicted outcomes

## Hypothesis H1: Performance differs with tDCS dose.

Based on the observed positive effect of 4 mA tDCS, we predict three possible outcome scenarios in the planned experiment with regard to hypothesis H1: 1. that there is a linear relationship between performance (measured as mean NCS throughout the initial task) and current intensity; 2. that there is a saturating but monotonic relationship, where the outcomes of 6 mA and 4 mA are not significantly different, but higher than that of 0 mA; or 3. that there is in fact a non-monotonic relationship as observed in the preliminary data, where the increase in intensity from 4 mA to 6 mA has a reversing effect. In order to detect any of these dose effects, we will apply a linear model with the current intensity group as a categorical fixed effect variable. We found through an online experiment (see below: “Predicting motor learning ability”) that a subject’s typing speed is positively correlated with the mean NCS throughout the learning task ($r = 0.62$). Therefore, our linear model will adjust for typing speed as a contributing factor, which we expect to improve statistical power. We powered the experiment at 80% by determining sample size through simulations of each of the three predicted outcome scenarios. 1,000 randomized iterations were run in MATLAB for each scenario, repeated over increasing sample sizes from 1 to 60 (Fig. 5a). Using the Lilliefors test on the prior data, we determined that the mean number of correct sequences during the initial task was normally distributed in the 0, 4,

and 6 mA groups. The typing speeds collected from the online experiment were likewise normally distributed. Thus, simulation data were randomly drawn from a joint normal distribution using the covariance between NCS from the preliminary data and typing speeds from our online experiment. These data assume the previous effect size of Cohen's d = 0.56 between the 0 and 4 mA conditions[49]. For the linear case, we extrapolated the mean value to the 6 mA group (d = 0.92). For the saturating case we used the same mean values for 4 mA and 6 mA, and for the non-monotonic case we use the same exact values from the preliminary data (Fig. 4A, concurrent stim.). A "successful" outcome was defined as one with a p-value less than α = 0.05 from an F-test on the model, indicating a significant effect of tDCS dosage. The statistical power for each sample size was thus determined by taking the percentage of successful simulations (Fig. 5b). At N=40, even without the typing speed covariate, the statistical power of our model is close to 80% in all three predicted scenarios. When adjusted for typing speed, the statistical power is over 90% in all three scenarios.

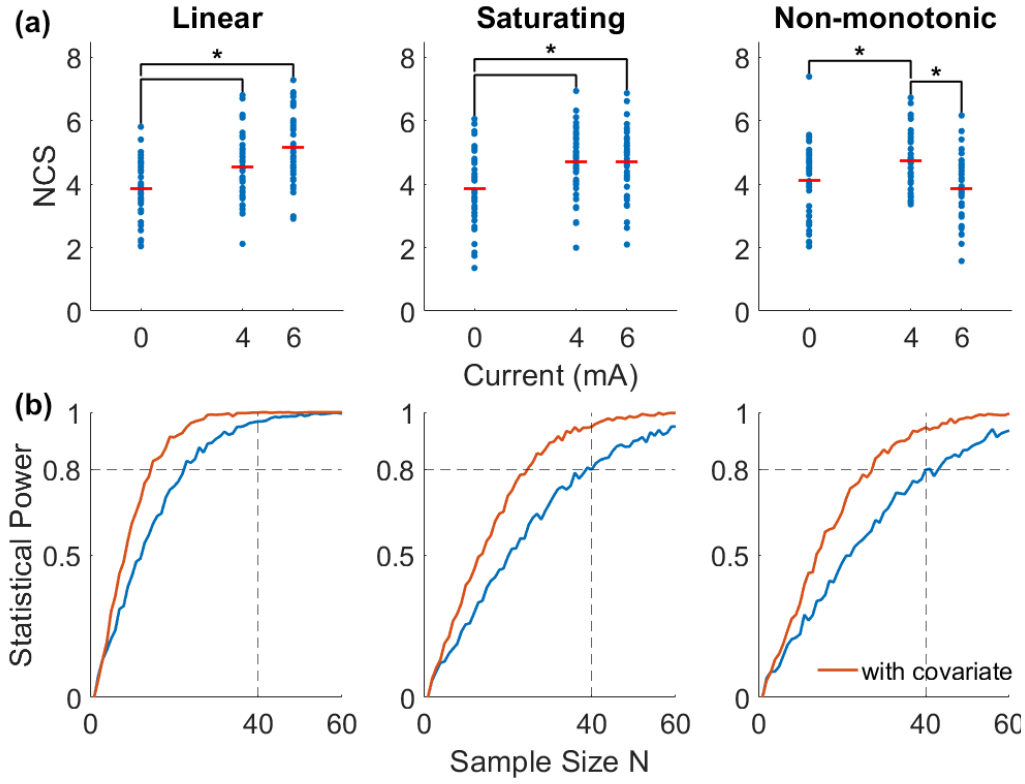


**Figure 5. Simulation of possible behavioral dose responses. (a)** An example of simulated results with N=40 in each group. Samples are jointly distributed based on the covariance between NCS from the preliminary data and typing speed from the online experiment. Mean values for 6 mA in the first scenario were linearly extrapolated from the preliminary data. Mean values for the second scenario were set with equal values for 4 mA and 6 mA. Mean values for the third scenario were set equal to those from the preliminary data. Brackets indicate significant differences (α = 0.05) found in post-hoc Tukey HSD. **(b)** Estimated statistical power of yielding p < 0.05 from an F-test on the linear model without (blue) and with (red) the typing speed covariate, across 1,000 simulations, calculated for sample sizes from 1 to 60. The desired threshold was set at 80% power.

## Hypothesis H2: Monotonic increase of MEP change with tDCS dose.

We will test for an effect on excitability by fitting a linear model on the post/pre MEP ratio with tDCS intensity as a graded fixed effect. Using G*Power[97], we determined that with N=40, even a modest effect of tDCS on MEP change with partial $\eta^2 = 0.12$ (relative to $\eta^2 = 0.91$ found by Ahn and Frohlich[67]) is sufficient to yield 95% power for hypothesis H2 ($\alpha = 0.05$). A significant finding for H2 would indicate a linear effect of tDCS intensity on MEP, whereas a null finding would suggest a nonlinear effect or no effect on MEP.

## Hypothesis H3: Performance correlates with change in MEP.

As power analysis for hypothesis H3 we simulated N=40 samples per group following the causal model presented in Fig. 1. We assumed a linear effect of tDCS-induced electric field onto the common learning-related neural substrate (arrow into white box in Fig. 1, denoted here as '*a*'). This common cause in turn linearly affects MEP and performance (denoted '*b1*' and '*b2*' here; we set *b1=b2=b* in the simulation). We added normally distributed observation noise (with unit standard deviation) to both outcome variables, plus a random offset per subject. We fit a linear mixed effects model for performance with MEP as a fixed effect and subjects as a random effect, then repeated the simulation 1,000 times to estimate power, i.e. the likelihood of obtaining a significant effect. Increasing the strength of *b* increases the correlation between performance and MEP. Thus, the Pearson correlation coefficient serves as a measure of effect size. With a significance threshold of $\alpha = 0.05$, we expect to observe a significant association between performance and MEP with 90% power at approximately $r = 0.29$ (Fig. 6). This estimate is valid for linear effects (*a, b1*, *b2*, with *b1=b2*) with no direct effects of tDCS on MEP or direct effects between MEP and performance. However, a significant association is also possible for non-linear common-cause effects (*b1*, *b2*) provided they are collinear (i.e. the nonlinear dependence on stimulation intensity has the same form). A null finding for H3 may rule out a linear/collinear common-cause effect, but it is also possible that direct effects cancel a common cause. Finally, a positive result is also possible due to electrical fields effects via separate mechanisms (direct arrow from field to MEP in Fig. 1). We do not expect H3 to hold if H1 and H2 show no effects. In short, a positive finding for H3 is consistent with, but does not prove a common physiological substrate for H1 and H2. A null finding for H3 would make it more difficult to argue that there is a common cause, but does not rule it out. Only direct observation of the neural substrate can answer this question.

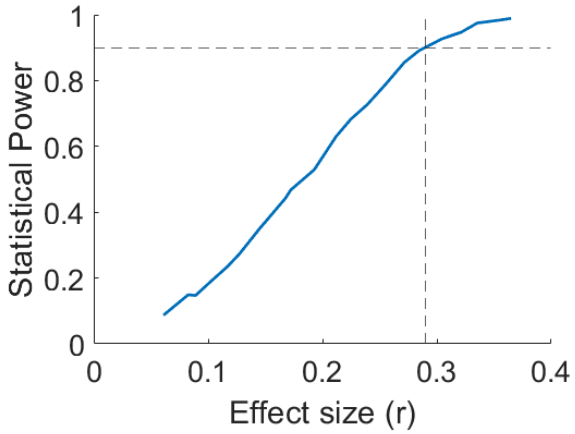


**Figure 6. Simulation of a common neural substrate with linear effects on both performance and MEP.** N=40 samples per group of MEP and performance measurements were randomly generated based on the model presented in Fig. 1. We assumed a linear effect *a* of electric field intensity on a neural substrate that in turn has equal linear effects *b1=b2=b* on the outcome measures. Since the Pearson correlation coefficient r between MEP and performance is proportional to *b*, we use r as a measure of effect size. Across 1,000 simulations we find that we can detect a significant association with 90% power when there is a correlation of at least r = 0.29.

## Sensation confound

We were also concerned that a potential tDCS dose response may be confounded by a sensation effect. Without matched controls, a positive correlation between physiological or behavioral outcomes and skin sensation could suggest that the tDCS effect is at least partly driven by a sensation placebo effect. Conversely, a negative correlation could suggest that stronger sensations caused by higher intensity stimulation may have a detrimental effect when the subject is more distracted during training. From our preliminary data we observed slightly higher skin sensation levels in the 6 mA group than in the +4 and -4 mA groups (Fig. 4b). We fit a linear mixed effects model over the initial task performance (number of correct sequences) with a fixed effect of sensation and random intercept of groups, excluding the 0 mA group. The estimate of the sensation fixed effect was β=-0.063, with p=0.17 and 95% CI between -0.15 and 0.026, suggesting no effect of sensation on performance. We will repeat this analysis on the final outcome to test whether there are any nonlinear effects due to variation in attention.

## Predicting motor learning ability

It is possible that some participants have generally better dexterous motor skills, resulting in better performance at the outset of the sequence training, or that they improve more quickly during training. Therefore, as an additional control for homogeneity in motor skill between groups, we will measure performance in a baseline task preceding the main trial (see Fig. 2c). We used the typing test described above. This task was followed by the same motor sequence learning task to be used in the main experiment, with the exact same sequence (4-1-3-2-4) that will be trained on when tDCS is applied simultaneously. 60 right-handed adults were recruited to complete this pilot experiment, through an online human subject research platform (Prolific,

London, UK). We found that the typing speed metric is positively correlated learning gain ($r(58) = 0.29$, $p = 0.024$; Fig. 7a) as well as baseline performance ($r(58) = 0.58$, $p = 1.2\times10^{-6}$; Fig. 7b) in our motor sequence learning task. To test whether the typing test may have a priming effect on the motor learning task, we conducted an additional online experiment with 60 right-handed adults who only performed the motor sequence learning task. Using a chi-squared test, we found that there was no difference in distribution of baseline performance during the initial typing test across groups ($\chi^2 = 4.5$, $p = 0.48$, N=60 per group; Fig. 7c). Based on these results, we determined that a short and simple typing test suffices as a baseline test to predict motor sequence learning ability, while being different enough from the main task such that task performance is not affected.

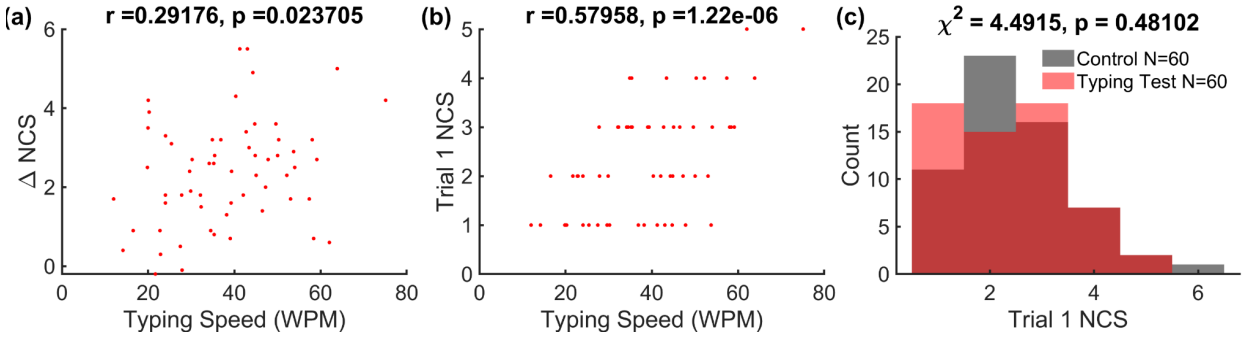


**Figure 7. Typing speed as a predictor of baseline motor skill and skill learning ability.** The same motor skill learning task was performed online on two cohorts of N=60 subjects, one with and the other without a preceding typing test. **(a)** Pearson correlation of performance gain (from first 10 trials to last 10 trials) with typing speed. **(b)** Pearson correlation of baseline skill performance during the first trial with typing speed. **(c)** Comparison of distributions (chi-squared test) of baseline skill performance in subjects who completed a baseline typing task beforehand and those who did not.

## Caveats

Except for a short typing task, the present study is identical to our previous study[49] in terms of behavioral demands to the subject. Nevertheless, the procedures add about 60 minutes at the start with the participant at rest. Any overall drop in performance between the initial 12-minute training session and the follow-up tasks after a 60-minute pause could be the result of fatigue. Additionally, the TMS during the pause could in theory affect task performance. Thus, this study is not properly designed to test for lasting effects or carryover effects. A failure to replicate our previous results on this could be a consequence of the intervening TMS or fatigue.

We are not aware of reports on lasting effects of 0.2 Hz single-pulse TMS, despite decades of research using this modality. We therefore do not not expect any interactions with the sequence learning task nor tDCS. At the same time, we cannot in theory rule out such interaction effects.

It is possible that the combination of tDCS with motor learning may saturate excitability. We should see in the control group whether the learning task on its own has any effect on MEPs and TEPs. In case of an effect in the control group, we would include this as a caveat that the study is not properly designed to isolate tDCS effects from motor learning alone. However, there

is some evidence that sequence learning tasks alone do not affect MEPs[54,55], as opposed to repeated strength exercises[18,50–53].

# Study Timeline

| | |
|---|---|
| 2023/10/06 | Study preregistration on OSF |
| 2023/10/09 | Data collection begins |
| | Blinded quality control in parallel with data collection |
| | Blinded coding for processing and analysis |
| 2024/01/11 | Submission of report to PCI RR (version 1.0) |
| 2024/01/30 | Submission of report to PCI RR (version 1.1) |
| 2024/03/07 | Report returned for revisions |
| 2024/04/09 | Submission of report to PCI RR (version 1.2) |
| TBD | Completion of data collection |
| TBD | Data processing/analysis and unblinding |

# Data and code availability

Deidentified data and analysis code will be made available upon completion of the study.

# CRediT author statement

**Gavin Hsu**: Investigation, Methodology, Formal analysis, Software, Project administration, Visualization, Writing - original draft. **Dylan J. Edwards**: Conceptualization, Methodology, Writing - review & editing. **Leonardo G. Cohen**: Conceptualization, Methodology, Writing - review & editing. **Lucas C. Parra**: Conceptualization, Data curation, Methodology, Formal analysis, Supervision, Writing - review & editing, Funding acquisition.

# Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: LP is listed as inventor in patents owned by CUNY, and has shares in Soterix Medical Inc.

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