Brain Stimulation 17 (2024) 553–560

Contents lists available at ScienceDirect

Brain Stimulation

journal homepage: www.journals.elsevier.com/brain-stimulation

Intensity-dependent effects of tDCS on motor learning are related to dopamine

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A B S T R A C T

Non-invasive brain stimulation techniques, such as transcranial direct current stimulation (tDCS), are popular methods for inducing neuroplastic changes to alter cognition and behaviour. One challenge for the field is to optimise stimulation protocols to maximise benefits. For this to happen, we need a better understanding of *how* stimulation modulates cortical functioning/behaviour. To date, there is increasing evidence for a dose-response relationship between tDCS and brain excitability, however how this relates to behaviour is not well understood. Even less is known about the neurochemical mechanisms which may drive the dose-response relationship between stimulation intensities and behaviour. Here, we examine the effect of three different tDCS stimulation intensities (1 mA, 2 mA, 4 mA anodal motor cortex tDCS) administered during the explicit learning of motor sequences. Further, to assess the role of dopamine in the dose-response relationship between tDCS intensities and behaviour, we examined how pharmacologically increasing dopamine availability, via 100 mg of levodopa, modulated the effect of stimulation on learning. In the absence of levodopa, we found that 4 mA tDCS improved and 1 mA tDCS impaired acquisition of motor sequences relative to sham stimulation. Conversely, levodopa reversed the beneficial effect of 4 mA tDCS. This effect of levodopa was no longer evident at the 48-h follow-up, consistent with previous work characterising the persistence of neuroplastic changes in the motor cortex resulting from combining levodopa with tDCS. These results provide the first direct evidence for a role of dopamine in the intensity-dependent effects of tDCS on behaviour.

1. Introduction

Electrical brain stimulation approaches - such as transcranial direct current stimulation (tDCS) - can modulate cortical functioning and affect behaviour [1]. An early example of stimulation influencing behaviour came from facilitatory effects of motor cortex tDCS on motor sequence learning [2]. This classic finding for the field, however, does not always replicate in all populations [3] and at times, stimulation has been shown to disrupt learning [4]. One possible reason for these inconsistencies is the use of varied stimulation protocols, driven by the fact we do not fully understand how stimulation modulates the brain. Indeed, it has recently been suggested that the some dosages (intensity) of stimulation applied to the motor cortex (0.7–1 mA) may not be enough to produce reliable effects, and indeed intensities as high as 4 mA may be required to achieve this [5,6].

The dose-dependence of tDCS effects on behaviour is incompletely understood. Whilst some studies suggest a linear relationship between stimulation intensity and effect of stimulation on behaviour [6,7], other studies suggest a non-linear effect [8–10]. Yet other studies fail to show any behavioural difference between different stimulation intensities [11,12], even when concurrently measured neurophysiological markers demonstrate dose-dependency [3,13]. Whilst the finding that dose effects vary between targeted brain regions and associated cognitive processes is perhaps not surprising, more research is necessary to elucidate the neural mechanism giving rise to such effects of tDCS on behaviour.

One possible mechanism of action for tDCS dose-dependency on learning is the modulation of dopamine levels in the cortico-basal ganglia pathways. Indeed, there is evidence that tDCS may alter striatal activity [14,15] and midbrain dopamine release [16–19], as pharmacological manipulations of dopamine strongly modulates the effects of non-invasive brain stimulation [20–24]. For example, levodopa combined with 2 mA motor cortex tDCS resulted in a 20-fold increase in the persistence of tDCS-induced neuroplasticity [20]. In addition, when tested against a fixed tDCS intensity, different doses of dopamine drug manipulations have non-linear effects on tDCS-induced neuroplasticity: only medium (and not small or large) doses of dopamine drugs have been found to alter the neuroplastic effects of tDCS [21,23–25]. This suggests that neuroplastic effects of tDCS might depend on a sweet spot of brain dopamine levels.

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https://doi.org/10.1016/j.brs.2024.03.015
Received 2 October 2023; Received in revised form 29 February 2024; Accepted 18 March 2024
Available online 9 April 2024

If different intensities of tDCS elicit different dopamine responses in the brain, and if intensity-dependent tDCS effects on behaviour result from different dopamine responses, then dopamine drug manipulations on behaviour should interact with tDCS intensity – which has not been systematically assessed to date. Thus, here, in a pre-registered study (https://osf.io/jegsr), we explored whether different intensities of tDCS alters behaviour partly by soliciting different brain dopamine responses. We used a motor sequence learning paradigm as our model, given the recent debate about optimal dosages of stimulation for this task [3,6,26]. First, we hypothesized that the effect of M1 tDCS on explicit motor sequence learning depends on tDCS intensity, anticipating that 4 mA in particular would facilitate learning. Second, we hypothesized that the effect of M1 tDCS on explicit motor sequence learning depends on brain dopamine levels, and thus the administration of levodopa would modulate effects of M1 tDCS on sequence learning. We anticipated that this effect would depend on tDCS stimulation intensity. Third, given one previous finding showing associations between dopamine genotypes and explicit motor sequence learning in older adults [27], we hypothesized that motor sequence learning may depend on brain dopamine levels. Thus, we predicted that in the absence of stimulation (sham stimulation, see below), increasing dopamine availability would modulate motor sequence learning.

2. Materials and method

2.1. Design

In this pre-registered study (https://osf.io/jegsr), we employed a factorial design, with between-subjects variables of dose (sham, 1 mA, 2 mA, 4 mA) and drug (levodopa, placebo), and within-subjects variables assessing repeating versus random sequences, as well as the different study blocks. Briefly, all participants were asked to execute a repeating or a random sequence across baseline, training, test, and retention blocks (see Fig. 1). Participants were randomly assigned to one of the eight conditions as shown in Table 1. In all sessions participants completed a 5-element sequence learning task, described below and outlined in Figs. 1 and 2.

2.2. Participants

Participants were right-handed and aged between 18 and 35 years old (mean age = 20 years, SD = 4 years), with no known neurological or psychiatric conditions and no contraindications to brain stimulation or Levodopa. In addition, as sequence learning in experts is differentially altered by tDCS compared to non-experts [28], participants were required to have fewer than 13 years of musical training, and currently engaging in no more than 20 h of musical training or gaming per week. Participants were pseudorandomly allocated to the following groups (see Table 1): levodopa sham tDCS (n = 17), levodopa 1 mA (n = 16), levodopa 2 mA (n = 19), levodopa 4 mA (n = 18), placebo sham tDCS (n = 17), placebo 1 mA (n = 17), placebo 2 mA (n = 17), placebo 4 mA (n = 16). The study was approved by the Human Research Ethics Committee at The University of Queensland and conformed to the Declaration of Helsinki. All participants provided written informed consent.

We adopted an adaptive Bayesian sampling plan. Our stopping rule stipulated that we were to recruit participants until a $BF_{10} > 3$ or $BF_{01} > 3$ was established for the critical hypothesis tests, providing moderate evidence for the alternative or null hypothesis for an effect of dose, or until we collected 30 complete datasets for each condition (total N = 240), whichever was sooner. Specifically, we tested for evidence ***of*** a dose-dependent effect of tDCS on explicit motor learning, by examining if a Bayesian *t*-test on sequence-specific learning shows moderate evidence (Bayes Factor >3) **for a difference between any two of the three dosages**, or if there was moderate evidence ($BF_{incl} > 3$) for including the main effect of dose in the Bayesian ANOVA. We tested for evidence ***against*** a dose-dependent effect of tDCS on explicit motor sequence learning by examining if Bayesian t-tests on sequence-specific learning showed moderate evidence ($BF_{01} > 3$) against differences between all of the three dosages, or if there was moderate evidence ($BF_{excl} > 3$) for excluding the main effect of dose in the Bayesian ANOVA. BF values were tested once a minimum of 15 participants were tested for

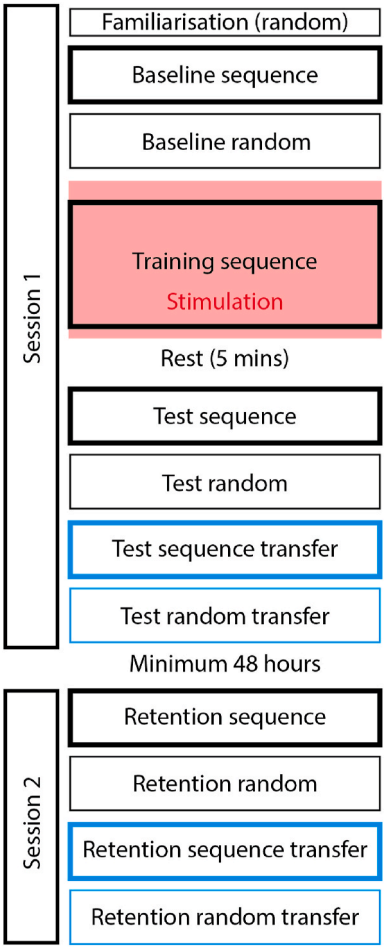

Fig. 1. Task structure for session 1 and session 2. On session 1, participants executed a 5-element repeating sequence, or a 5-element random sequence at baseline, training, and test, using their left hand. In transfer blocks, they executed sequences with their untrained right hand. Stimulation occurred during training, After a minimum 48 h interval, participants returned for session 2, where retention was first assessed for the trained hand, and then for the untrained hand (transfer blocks).

Table 1
Participants were randomly allocated to one of the 8 study conditions, where they received a placebo or levodopa tablet, and received either sham, 1 mA, 2 mA, or 4 mA tDCS during the training block of the task. Participant numbers are after exclusions (a total of three).

Drug	Stimulation: Sham	1mA	2mA	4mA
Placebo	N = 17	N = 17	N = 17	N = 16
Levodopa	N = 17	N = 16	N = 18	N = 16

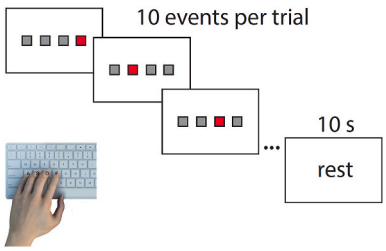

Fig. 2. During the sequence learning task, participants saw 4 grey boxes on-screen which corresponded to the four finger positions on the keys ASDF. During sequence trials, participants executed a 5-element sequence (FSDFA) by responding as quickly and as accurately as possible to the red target stimulus, which appeared on-screen according to the sequence order. In random trials, the red target stimulus appeared in random order. During the training block, participants executed 28 bins of 10 trials: each bin of 10 trials was followed by a 10 s rest break. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

each condition. We ended up with a sample size of between n = 16 to n = 19 per condition (total: n = 138). As per our pre-registration, we then discarded 3 datasets from the analyses due to poor task performance (accuracy less than 60% across training and/or transfer blocks of the experiment), with the included participant numbers show in Table 1. Specifically, two participants were removed from the levodopa 4 mA group, and 1 participant from the levodopa 2 mA group.

2.3. Drug and stimulation manipulations

In session 1, participants first completed blood pressure and mood assessments, and then received either placebo (vitamin) or levodopa (Madopar 125: 100 mg levodopa and 25 mg benserazide hydrochloride), crushed and dispersed in orange juice. An experimenter uninvolved in data collection prepared the solution. This procedure was sufficient to achieve double blinding in previous work [29,30]. tDCS electrodes were then set up, before participants completed a second blood pressure and mood rating assessment, and a brief questionnaire assessing chronotype (the Morningness–Eveningness Questionnaire, DMEQ), which has recently been shown to modulate responsivity to brain stimulation and performance on motor sequence learning tasks [31].

Participants then started the task component of the session (see Fig. 1). They completed the familiarization and the baseline blocks of the sequence learning task. Stimulation commenced – approximately 30 min after drug ingestion – within the window of peak plasma availability [32]. Upon stimulation commencement, participants completed the training block, which assessed acquisition of the sequence. Stimulation lasted for 10 min, which coincided with the completion of the training block. After a 5-min rest break during which electrodes were removed, participants completed an end-of-acquisition block. Blood pressure and mood rating assessment were again completed (approximately 1 h after drug ingestion). After an overnight interval (minimum 48 h, sufficient for effects of the drug and the stimulation manipulations to dissipate), participants returned to the lab for a no-drug, no-stimulation follow-up session.

For the 1 mA, 2 mA, 4 mA conditions, stimulation lasted for 11 min (30 s ramp up, 10 min constant, and 30 s ramp down). For the sham condition, the parameters were the same but the stimulation lasted for 1 min 15 s (stimulation intensity was evenly split between 1, 2, and 4 mA) followed by regular small test 'pulses' to maintain some sensation and allow impedance values to be calculated and displayed. Whether stimulation was active or sham was double blinded using stimulation codes provided by a team member who did not participate in data collection. Stimulation intensity was only single blind, due to limitations of the stimulator. Stimulation was delivered via a NeuroConn stimulator with two 5 × 5 cm electrodes. We had participants complete the explicit sequence learning task with their non-dominant left hand, as the non-dominant hand is thought more sensitive to training related performance improvements [33]. Thus, we targeted the contralateral right M1 hemisphere with stimulation, consistent with previous work that examined effects of M1 tDCS on explicit sequence learning [34,35]. Specifically, the target electrode (5 cm × 5 cm) was placed over the EEG 10–20 location C4 [36], and the reference electrode (5 cm × 7 cm) was placed over the contralateral supraorbital region (the area above the left brow ridge; see Fig. 3). Both electrodes were encased in saline-soaked sponge pads with a layer of highly conductive saline gel (SignaGel) [37].

2.4. Task

Participants were first asked to place their little, ring, middle, and index fingers of their left hand on the keys ASDF. A row of four boxes were presented on screen, three grey and one red target stimulus, each corresponding spatially to the finger positions. The participants' task was to respond as quickly as possible to the red target stimulus by pressing the appropriate key: in each trial, participants executed 5 keypresses according to the spatial location of the red target. There were two types of trials: sequence trials and random trials. In each sequence trial, the grey boxes turned red 5 times according to the 5-element tap sequence for the sequence blocks [FSDFA]. The sequence was the same throughout all trials for all participants. In each random trial, the grey boxes turned red 5 times in random order. At the start of each trial, brief prompts denoted trial commencement: "ready? (300 ms) "start!" (300 ms duration). No performance feedback was presented throughout the entire study.

First, participants were given the following on-screen instructions, which were read out loud by the experimenter "In this study, your task is to respond using the below keys when the corresponding box is highlighted red, using your LEFT hand. Press any key to start the familiarization block". Then, participants completed a familiarization block (3 trials) with a random sequence to familiarise them with the task. Upon completing the familiarization block, participants were given the following on-screen instructions, which were read by the experimenter to ensure participant comprehension: "In some trials, the cues will appear in a fixed sequence. Respond to the cues as QUICKLY and ACCURATELY as possible."

After familiarization, participants completed the baseline sequence block (10 trials, i.e., 10 executions of the 5-element sequence), followed by a baseline random block (10 trials). Stimulation commenced and was maintained for 2 min, before the start of the training block, which assessed acquisition of the repeating sequence. The training block consisted of 280 sequence trials (or 28 bins), during which a timed 10 s rest break was presented after every 10 trials. The training block was followed by a 5-min rest break, during which the stimulation ended and the electrodes were removed. Participants then completed the end of acquisition block (i.e., the test block), which consisted of 1 sequence bin

Fig. 3. tDCS montage, with the 5 cm × 5 cm anode placed over the C4, targeting the right primary motor cortex, and the reference electorde (5 cm × 7 cm) placed over the left supraorbital area.

and 1 random bin (10 trials for each bin) with the training hand, and 1 sequence bin and 1 random bin for the untrained right hand. This concluded the first session (Session 1).

After an interval of minimum 48 h (sufficient time for effects of tDCS and levodopa to wash out), participants returned to the lab for the follow-up session (Session 2), during which they completed the retention sequence block and the retention random block (50 trials each), followed by an intermanual transfer test, during which they completed the retention sequence block and the retention random block (50 trials each) with their untrained right hand.

Blinding efficacy for both the experimenter and the participant were assessed at the end of the first session, by asking the participant to complete a questionnaire which asked whether they thought they received (1) placebo or Madopar, and (2) sham, 1 mA, 2 mA, or 4 mA stimulation. The experimenter also completed questionnaires assessing whether they thought the participant received (1) placebo or Madopar, and (2) sham or true stimulation.

2.5. Statistical analyses

Reaction times from correct keypresses were used to estimate ***sequence-learning*** (also subsequently referred to as learning). We accounted for individual differences in reaction times by normalising reaction times to repeating sequences relative to reaction times in response to random sequences at baseline, prior to stimulation (i.e., from the baseline). Specifically, sequence learning was calculated by subtracting reaction times mean-averaged from sequence bins from reaction times mean-averaged from the baseline random bins (i.e., baseline mean random reaction time), divided by baseline mean random reaction time. This is expressed as follows: [(mean random reaction time – mean repeat reaction time)/mean random reaction time] [38]. Higher values indicate greater sequence-specific improvement. Sequence learning was quantified for baseline, acquisition, end of acquisition, retention, and intermanual transfer. We also quantified participants chronotypes via scores from the Morningness–Eveningness Questionnaire, and time of day (AM versus PM). These have not been included in the analyses as we met our stopping criteria with a sample we felt was too small to include covariates.

As per our pre-registration, we utilised Bayesian analyses. This allowed for a relatively conservative statistical approach, and quantification both for and against experimental effects. Specifically, Bayesian ANOVAs and t-tests were run using JAMOVI, version 2.3.28.0. For the training session, we ran Phase (p1, p2, p3, p4) x Bin (1 ….7) ANOVAs for sequence specific learning, calculated for each of the 28 training bins. For the no-drug, no-stimulation follow-up session, we ran Phase (p1, p2) x Bin (1 … 5) ANOVAs for sequence specific learning for the retention block and for the transfer block.

Bayes Factors (BF) values of 1–3 were interpreted as anecdotal, BF of 3–10 as moderate, and BF > 10 as strong evidence for the test hypothesis/variable inclusion (BF_{10} or BF_{incl}), or for the null hypothesis/variable exclusion (BF_{01} or BF_{excl}). BF values ~1 were interpreted as providing no evidential value. Statistical analyses were conducted using JAMOVI version 2.3.18.0.

3. Results

3.1. Baseline

Sequence specific learning did not differ across stimulation conditions prior to the start of the training phase and application of stimulation (main effect of Intensity, BF_{excl} = 15.244, Intensity × Drug interaction, BF_{excl} = 6.807). We found indeterminate evidence for the main effect of drug (BF_{excl} = 0.673).

3.2. Sequence learning

Overall, training improved performance. Specifically, reaction times for the sequence trials reduced pre-to post-training ($BF_{10,u}$ = 3.49 e+55, cohen's d = 2.24, 95% CI [2.02, 2.51]), in contrast to random trials ($BF_{10,\ u}$ = 0.145, cohen's d = 0.09, 95% CI [−0.10 0.29]), as Time (Pre-training, Post-training) x Sequence (Sequence, Random) ANOVA showed a Phase × Sequence interaction (BF_{incl} = 2.48E+30). These benefits were retained after the >48 h delay, as reaction times for the first sequence bin remained faster than baseline ($BF_{10,u}$ = 2.70 e+42, cohen's d = 1.90, 95% CI [1.68 2.14]). A summary of the overall reaction times results is shown in Fig. 4. Training-related performance improvements in sequence specific learning was evident across each training phase (main effect of phase, BFincl = ∞) as well as across bins (main effect of bin, BFincl = 3.52e+52).

3.3. Stimulation and levodopa modulated acquisition

Overall, in the absence of levodopa, 4 mA tDCS improved – and 1 mA tDCS impaired – acquisition of the sequence during training. The beneficial effect of 4 mA tDCS was reversed with the administration of levodopa (see Fig. 5). Indeed, acquisition across training phases was differentially altered by stimulation dose (Phase × Intensity interaction BF_{incl} = 173.690) as well as by the drug manipulation (Phase × Drug interaction $BF_{\ incl}$ = 18284.715). Follow-up Intensity (sham, 1 mA, 2 mA, 4 mA) x Phase (Phase 1 … 4) x Bin (1 … 7) Bayesian ANOVAs run for the placebo group showed a Phase × Intensity interaction (BF_{incl} = 3.080), which was driven by improved acquisition for 4 mA [(4 mA vs sham: $BF_{10,\ U}$ = 12.131; 4 mA vs 1 mA: $BF_{10,\ U}$ = 1.14E+07; 4 mA vs 2 mA: $BF_{10,\ U}$ = 19.697), and impaired acquisition for 1 mA (1 mA vs sham: $BF_{10,\ U}$ = 8.9667, 1 mA vs 2 mA: $BF_{10,\ U}$ = 5.1313, 1 mA vs 4 mA: BF10 = 1.14E+7). 2 mA tDCS did not alter acquisition compared to sham (evidence for the null, $BF_{01,\ U}$ = 13.583). The influence of levodopa on acquisition depended on stimulation intensity, (see Fig. 6 left), as a follow-up Intensity x Phase x Bin ANOVA run for the levodopa group showed a Phase × Intensity interaction, BF_{incl} = 3.080. With levodopa, the 4 mA group showed worse acquisition than the 2 mA and sham groups (4 mA vs sham: $BF_{10,\ U}$ = 5.626; 4 mA vs 2 mA: $BF_{10,\ U}$ = 615.994), and similar acquisition as 1 mA (moderate evidence for the null: 4 mA vs 1 mA: $BF_{01,\ U}$ = 6.096). 2 mA tDCS improved acquisition compared to 1 mA ($BF_{10,\ U}$ = 7.676), but not compared to sham (moderate evidence for the null, $BF_{01,\ U}$ = 4.050). The 1 mA group showed similar learning as sham and 4 mA, as shown by moderate evidence for the null (1 mA vs sham: $BF_{01,\ U}$ = 3.410; 1 mA vs 4 mA: $BF_{01,\ U}$ = 6.09).

To quantify the effect of levodopa for each of the four intensities, we ran exploratory Drug x Phase x Bin ANOVAs (sham, 1 mA, 2 mA) separately for each intensity. Whilst the 4 mA group showed impaired

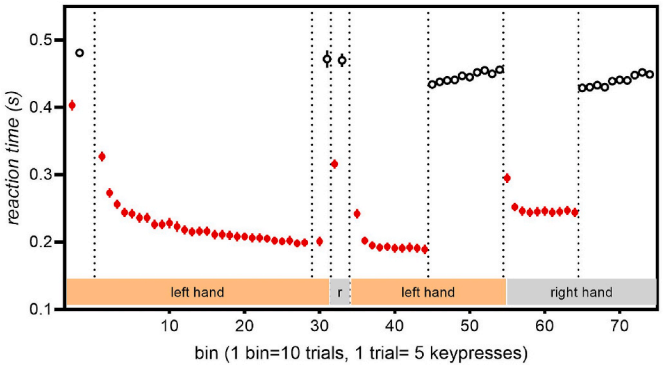

Fig. 4. Reaction times for correct keypresses throughout all study phases. Red symbols = sequence trials, clear symbols = random trials. Lower values indicate faster responses. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

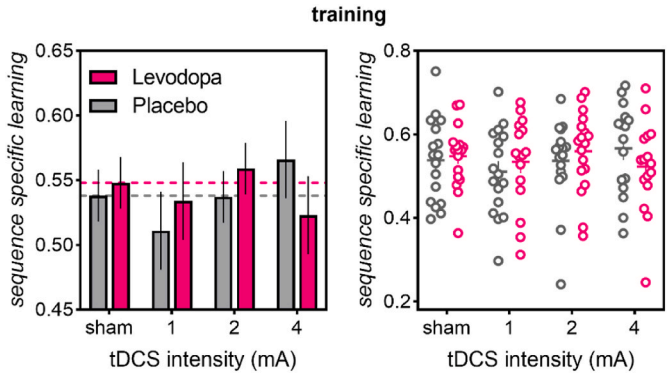

Fig. 5. Sequence-learning during the training block for average sequence specific learning (left) and the individual data points (right). Higher values indicate greater sequence-learning. Error bars (left) indicate standard error of the mean. In the placebo group (grey bars), 4 mA stimulation improved performance compared to all other groups, whereas 1 mA stimulation resulted in worse performance than all other groups. In the levodopa group (pink bars), 4 mA stimulation resulted in worse-than-sham performance, whereas 2 mA stimulation resulted in better-than-sham performance. Dotted lines indicate performance for the sham stimulation group. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

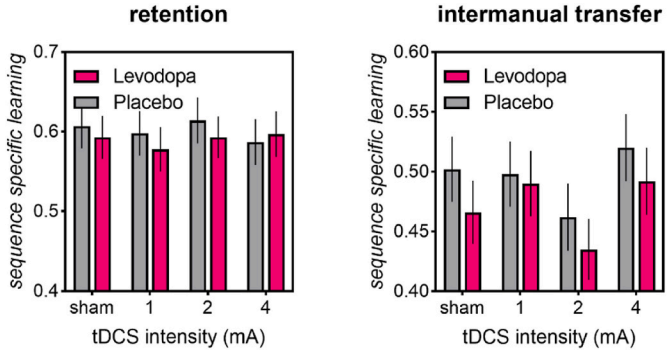

Fig. 6. Sequence specific learning at follow-up, >48 h after the training session. Whilst retention block performance was similar across stimulation and drug conditions, intermanual transfer assessed at follow-up showed poorest intermanual transfer for the 2 mA condition for both the placebo group (grey bars) and the levodopa group (pink bars). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

acquisition with levodopa than placebo [$BF_{10,\ U} = 3827$], the 1 mA and the 2 mA groups tended to show improved acquisition with levodopa than placebo, as shown by weak-moderate evidence for an effect of levodopa [1 mA: $BF_{10,\ U} = 2.42$, 2 mA: $BF_{10,\ U}$ for $= 3.61$]. For sham tDCS, levodopa did not alter acquisition [moderate evidence for the null, $BF_{01,\ U} = 6.61$].

3.4. End of acquisition

Stimulation intensity and levodopa did not affect performance at the end of acquisition, as shown by moderate evidence for the null hypothesis (main effect of intensity: $BF_{excl} = 8.849$, main effect of Drug: $BF_{excl} = 3.846$, Drug × Hand interaction: $BF_{excl} = 5.208$, Drug x Hand × Intensity interaction $BF_{excl} = 9.523$).

3.5. Retention and intermanual transfer at follow-up

Retention. At follow-up, retention of sequence learning was evidenced in better-than-naive performance when comparing the 10 retention bins to the first 10 training bins ($BF_{10,\ U} = 2.65e{+}155$). Neither levodopa nor stimulation dose altered retention (main effect of Drug, $BF_{excl} = 2.510$, main effect of Intensity: $BF_{excl} = 3.956$, Drug × Intensity interaction: $BF_{excl} = 3.460$, Drug × Phase interaction, $BF_{excl} = 9.509$, Intensity × Phase interaction, $BF_{excl} = 8.944$). The absence of an effect of levodopa on tDCS effects at 48-h follow-up is consistent with previous findings that levodopa augments the persistence of motor cortex tDCS effects on motor evoked potentials up to 48 h (and not beyond 48 h) after stimulation cessation [20].

Intermanual transfer. Stimulation intensity at training altered intermanual transfer at follow-up. Whilst the main effect of intensity was inconclusive ($BF_{incl} = 0.674$), t-tests showed that across both the placebo and the levodopa group, 2 mA tDCS resulted in poorest intermanual transfer compared to all other stimulation conditions (very strong evidence for the test hypothesis: 2 mA vs sham: $BF_{10,\ U} = 98.343$, 2 mA vs 1 mA: $BF_{10,\ U} = 14176.472$, 2 mA vs 4 mA, $BF_{10,\ U} = 1.88e{+}7$).

Levodopa at training did not alter intermanual transfer: we found inconclusive-to-moderate evidence for excluding the effect of the drug manipulation (main effect of Drug: $BF_{excl} = 1.440$, Drug × Intensity interaction, $BF_{excl} = 8.264$).

3.6. Blinding efficacy

Overall, blinding was successful for both experimenters and participants. Specifically, experimenters were not above chance at correctly guessing whether participants were assigned to the levodopa or placebo condition, or the sham versus active stimulation condition, as Bayesian binomial tests showed moderate evidence for the null hypothesis (drug condition: $BF_{01} = 3.591$, stimulation condition: $BF_{01} = 5.445$). Participants were not above chance at correctly guessing the drug condition ($BF_{01} = 9.151$) and not above chance at correctly guessing which one of the four stimulation conditions they were assigned to ($BF_{01} = 8.669$). We additionally recoded participants guesses for the stimulation condition to be binary, where guesses of 0 mA was considered to be guesses of the sham condition, and guesses of 1 mA, 2 mA and 4 mA condition was recoded as active stimulation. Overall, there was a bias for participants to report they received active stimulation – in line with previous similar reports (e.g. Ref. [10]) – and in fact similar proportions of active vs. sham responses were found in all groups. Specifically, the sham group were greater than chance at incorrectly responding that they received active stimulation ($BF{+}0 = 6.548e{+}11$), as 22 out of the 33 participants in the sham group responded that they received active stimulation. The sham group tended to be lower than chance at correctly guessing that they received sham stimulation (anecdotal-moderate evidence, $BF{+}0 = 2.939$) as only 4 of the 33 sham group participants correctly responded that they received sham stimulation). The active stimulation groups were greater than chance at incorrectly responding that they received sham stimulation ($BF0{+} = 7.793$): 22 out of 99 participants from the three active stimulation groups responded that they received sham stimulation. The active stimulation groups were not greater than chance at correctly guessing that they received active stimulation (anecdotal evidence for the null hypothesis, $BF0{+} = 2.730$), where only 77 out of 99 of the active stimulation group participants responded correctly that they received active stimulation.

4. Discussion

In a pre-registered, double-blind study, using conservative Bayesian statistics, we found an intensity-dependent effect of anodal motor cortex tDCS on explicit motor learning that interacted with exogenous dopamine. Specifically, in the absence of levodopa, 1 mA impaired, 2 mA had no effect, and 4 mA tDCS improved acquisition of motor sequence during training. Conversely, administration of levodopa reversed the beneficial effects of 4 mA tDCS on acquisition. To the best of our knowledge, this is the first study to demonstrate a causal role of dopamine in the intensity-dependent effects of tDCS on behaviour.

The benefit to learning in the highest intensity 4 mA condition suggests that higher doses of injected current can be more potent in modulating function. This result is consistent with previous findings of large benefits of 4 mA tDCS on sequence learning in neurotypical young adults [5,6]. Similarly, positive dose-response relationships have been suggested from meta-analyses on clinical populations such as those who have had a stroke [39] or major depression [40]. An important caveat however is that the effect of stimulation intensity is likely task-dependent. For example, whilst linear effects of intensities have been shown for mind-wandering [41], non-linear effects of intensity have been shown for working memory training [8] and multitasking training [10]. In a similar vein, the effect of stimulation intensity here depended on the stage of learning. Here, 4 mA tDCS selectively improved whilst 1 mA impaired acquisition without altering retention or intermanual transfer. In contrast, 2 mA tDCS selectively impaired intermanual transfer without altering acquisition or retention. This parallels other work which found that different stimulation intensities differentially altered training and transfer [10].

To date, there have been mixed findings for the efficacy of 1 mA anodal tDCS to modulate motor sequence learning. Several studies in the field indicated stimulation may enhance learning ([2,42]), but several studies have since found the reverse where performance is impaired (e.g. Refs. [4,43,44]. Whilst it is not clear why this variaibility in findings exist, it is possible that methodological differences (e.g. whether participants were informed of the sequence, and electrode montages, when stimulation was applied) may contribute.

We found that whilst levodopa resulted in a small benefit to acquisition of sequence learning when paired with lower tDCS intensities (1mA–2mA), levodopa reversed beneficial effects at the highest 4 mA tDCS intensity. By showing that levodopa modulates effects of tDCS intensity on sequence learning, we demonstrate a causal role of dopamine in the way stimulation influences learning. Frontal cortex tDCS is known to induce midbrain dopamine release [16–18] and – at the dose used here – levodopa primarily increases midbrain dopamine [45]. A large body of evidence demonstrates an inverted U-shaped relationship between brain dopamine and cognition [46], for a review, see [47]. Based on our findings showing that the effect of levodopa on sequence learning depends on the stimulation intensity used, it may be that dosage of tDCS differentially modulates dopamine release, which influences performance. Stimulation dose seems likely to modulate dopamine release: for example, moderate but not low or high intensities of repetitive transcranial magnetic stimulation resulted in dopamine release in the rat dorsolateral striatum [48]. According to this proposal, higher stimulation intensities might increase brain dopamine to optimal levels, but combining 4 mA tDCS with levodopa results in excessive dopamine, impairing performance. This possibility remains to be shown experimentally.

Contrary to our predictions, we did not find evidence for an effect of levodopa on motor sequence learning in the absence of active tDCS. We predicted an effect of levodopa on sequence learning, based on previous studies linking dopamine genotypes and explicit motor sequence learning in older adults (Schuck et al., 2013), as well as effects of levodopa on explicit sequence learning in Parkinson's disease e.g.,[49]. Profound differences in dopamine function between young and older adults for a review, see [50] and between older adults and Parkinson's disease patients [51] make it challenging to extrapolate expected findings across such populations. Our findings suggest that in young healthy adults, levodopa – at least with the dosage and the task used here – does not influence explicit motor sequence learning. It is noteworthy that recent work has similarly shown no effect of the dopamine D2 antagonist haloperidol on the learning of motor sequences in young adults: haloperidol only affected the speed of individual movements that comprised each sequence [52]. Future research is necessary to clarify if such findings will generalise to other populations with altered dopamine function.

Our findings have implications for the application of tDCS in populations with altered dopamine function, including older adults and Parkinson's disease. In such populations, there is an increasing body of work demonstrating either null effects [3,53,54] or unexpected negative effects of tDCS on behaviour [4,55,56]. For example, Ghasemian-Shirvan et al. [3] found no effect of anodal 1 mA, 2 mA, 3 mA motor cortex tDCS on motor sequence learning in elderly participants, even though a dose-dependent effect was prominent for motor evoked potentials. Stimulation applications in these populations may benefit from consideration of baseline dopamine function, potentially through pre-conditioning stimulation protocols, use of medications to modulate dopaminergic function e.g., [57, 58], and/or stimulation protocols designed to optimise outcomes specifically for these populations.

Previous imaging studies have shown that anodal motor cortex tDCS reduces concentrations of the inhibitory neurotransmitter Gamma-Aminobutyric Acid (GABA) in the contralateral and ipsilateral motor cortex [59], and the extent of this decrease correlates with amount of improvement in sequence learning [60]. tDCS to the frontal cortex is also known to result in a disinhibitory effect on the midbrain, by increasing striatal GABA levels [19]. Indeed the larger the striatal GABA increase, the larger the stimulation-induced dopamine release in the striatum [19]. Thus, interactions between the GABAergic and dopaminergic systems might give rise to the effect of tDCS on the cortex and behaviour. Emerging work has begun to combine manipulations of different neurochemicals to better understand how tDCS alters brain neurophysiology [61]. Future studies may combine measures/manipulations of GABA, dopamine, tDCS and stimulation dosage to disentangle and elucidate the combined contributions of these to behaviour.

It is important to note that whilst we show a non-linear dosage effect of 1 mA, 2 mA and 4 mA tDCS on explicit sequence learning in young neurotypical adults, we do not know if our findings will generalise to other populations. For example, older adults, who show age-related changes in brain dopamine function [62], and populations with altered brain dopamine function such as attention-deficit disorder [63]. In addition, our study held the dose of dopamine medication constant whilst varying tDCS intensity, in contrast to some previous studies which held stimulation dose constant whilst examining how systematically manipulating different dopamine drug dosages altered motor cortex excitability [23,25]. Whilst we found a group level effect of manipulating dopamine and different stimulation intensities, it is likely that there are individual differences in the way individuals respond to different doses of dopamine medications and different doses of tDCS (i.e., intensity, duration, frequency, etc). Future studies should take advantage of advancements in individualisation of brain stimulation protocols [64] to explore questions such as how individual differences in brain dopamine function relate to individual differences in optimal brain stimulation protocols. Additionally, we used an electrode montage that targeted the right M1 as participants trained with their non-dominant left hand. It is clear that pathways between the hemispheres contribute to acquisition of sequence learning [35], and different stimulation montages can differentially alter sequence learning [34]. Thus, it remains to be seen what the effect of tDCS dosage and levodopa would be from bilateral or ipsilateral stimulation.

In sum, we show non-linear dosage effects of tDCS on explicit motor sequence learning, and these interact with dopamine availability. This work represents a significant step forward in understanding how stimulation can modulate motor learning, giving mechanistic insights into neurochemical contributions to the efficacy of stimulation. Developments such as these will improve our ability to target stimulation to situations where it is more likely to be efficacious, and gives insight into how we may go about optimising applications in the future.

CRediT authorship contribution statement

Li-Ann Leow: Conceptualization, Data curation, Formal analysis,

Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Jiaqin Jiang:** Investigation, Project administration, Methodology. **Samantha Bowers:** Investigation, Methodology, Project administration. **Yuhan Zhang:** Investigation, Methodology, Project administration. **Paul E. Dux:** Conceptualization, Funding acquisition, Resources, Supervision, Writing – review & editing. **Hannah L. Filmer:** Conceptualization, Funding acquisition, Resources, Supervision, Visualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

We declare that none of the authors have any conflicts of interests that could have influenced the work.

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