Cooperation not competition: bihemispheric tDCS and fMRI show role for ipsilateral hemisphere in motor learning

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Cooperation not competition: bihemispheric tDCS and fMRI show role for ipsilateral hemisphere in motor learning

Short title: Role of ipsilateral hemisphere in motor learning

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Abstract

What is the role of ipsilateral motor and pre-motor areas in motor learning? One view supposes that ipsilateral activity suppresses contralateral motor cortex, and, accordingly, that inhibiting ipsilateral regions can improve motor learning. Alternatively, the ipsilateral motor cortex may play an active role in the control and/or learning of unilateral hand movements. We approached this question by applying double-blind bihemispheric transcranial direct current stimulation (tDCS) over both contralateral and ipsilateral motor cortex in a between-group design during four days of unimanual explicit sequence training in human participants. Independently of whether the anode was placed over contralateral or ipsilateral motor cortex, bihemispheric stimulation yielded substantial performance gains relative to unihemispheric or sham stimulation. This performance advantage appeared to be supported by plastic changes in both hemispheres. First, we found that behavioral advantages generalized strongly to the untrained hand, suggesting that tDCS strengthened effector-independent representations. Secondly, functional imaging during speed-matched execution of trained sequences conducted 48 h after training revealed sustained, polarity-independent increases in activity in both motor cortices relative to the sham group. These results suggest a cooperative rather than competitive interaction of the two motor cortices during skill learning and suggest that bihemispheric brain stimulation during unimanual skill learning may be beneficial because it harnesses plasticity in the ipsilateral hemisphere.

Significance statement

Many neurorehabilitation approaches are based on the idea that is beneficial to boost excitability in the contralateral hemisphere while attenuating that of the ipsilateral cortex to reduce interhemispheric inhibition. We observed that bihemispheric tDCS with the excitatory anode either over contralateral or ipsilateral motor cortex facilitated motor learning nearly twice as strongly as unihemispheric tDCS. These increases in motor learning were accompanied by increases in fMRI activation in both motor cortices that outlasted the stimulation period, as well as increased generalization to the untrained hand. Collectively, our findings suggest a cooperative—rather than competitive—role of the hemispheres and imply that it is most beneficial to harness plasticity in both hemispheres in neurorehabilitation of motor deficits.
Introduction

Even strictly unilateral motor behaviors—such as moving one hand—rely on interactions between the cerebral hemispheres. However, the nature of this interaction is incompletely understood (Perez and Cohen, 2009; Di Pino et al., 2014). One influential idea is the ‘interhemispheric competition’ model, according to which the two hemispheres mutually suppress each other via inhibitory interconnections (Curtis, 1940; Ferbert et al., 1992; Daskalakis et al., 2002; Chen, 2004; Ni et al., 2009). This notion has shaped theories about how best to improve motor learning through brain stimulation. It has been suggested that motor learning may be facilitated not only by exciting contralateral motor cortex, but also by inhibiting ipsilateral cortex, particularly in the context of stroke rehabilitation (Murase et al., 2004; Hummel and Cohen, 2006; Williams et al., 2010; Takeuchi et al., 2012). tDCS has been shown to increase motor-evoked potentials (MEPs) when the anode is placed above primary motor cortex (M1) (Nitsche and Paulus, 2000) and to decrease MEPs in the presence of a cathode (Nitsche et al., 2003). Consistent with the interhemispheric competition model, Vines et al. (2008) demonstrated that bihemispheric tDCS—with the cathode located over ipsilateral M1—improves performance more than unihemispheric tDCS.

However, there are reasons to doubt that the bihemispheric advantage is due to the suppression of ipsilateral cortex. While MEPs typically decrease under the cathode after unihemispheric stimulation (Nitsche et al., 2003), polarity-specific changes are reduced after bihemispheric stimulation (O’Shea et al., 2014). Thus, increased neural plasticity subsequent to tDCS may not be closely linked to polarity-specific excitability changes measured with TMS. Rather, plasticity increases may be attributable to the electrical current running transversely, rather than radially, through the cortical tissue (Rahman et al., 2013). If true, then tDCS effects should depend on the spatial current distribution, but not on current direction.

Under this assumption, bihemispheric tDCS may increase plasticity in both motor cortices, independently of polarity. Additionally, ipsilateral motor cortex may have an active role in the execution and learning of complex movements. Recent functional magnetic resonance imaging (fMRI) (Diedrichsen et al., 2013; Wiestler and Diedrichsen, 2013), and electrophysiological (Ganguly et al., 2009) studies have demonstrated that activity in M1—although often suppressed below baseline—contains information about ongoing ipsilateral movements. This activity could contribute to movement control either through directly descending ipsilateral projections, or by shaping activation patterns on the contralateral side (Verstynen et al., 2005). According to this ‘interhemispheric cooperation’ model, bihemispheric tDCS is more effective than unihemispheric stimulation, not because it silences ipsilateral cortex, but because it increases ipsilateral plasticity.
To adjudicate between these explanations, we tested the effects of reversing the polarity of bihemispheric stimulation. We trained 64 participants in a double-blind study, either using sham, unihemispheric, conventional bihemispheric, or reversed-polarity bihemispheric tDCS (Fig. 1a). Participants performed an unimanual sequence task with either left or right hand over four days (Fig. 1b). After training and a behavioral post-test without tDCS, participants underwent fMRI to elucidate the neural changes induced by stimulation.

The interhemispheric competition model (assuming polarity-specific tDCS effects, see Table 1 for other assumptions) predicts that reversed-polarity bihemispheric tDCS should impair performance compared to the sham group. Not only should contralateral cathodal stimulation suppress the motor areas involved in learning, but ipsilateral anodal stimulation should further increase interhemispheric inhibition. fMRI should reveal opposite changes in the hemisphere that received anodal and cathodal stimulation.

Contrastingly, the interhemispheric cooperation model (assuming polarity unspecific tDCS effects) predicts that plasticity is induced regardless of current direction, and that the behavioral advantage arises from plasticity in both hemispheres. Therefore, reversed-polarity tDCS should be as effective as conventional bihemispheric tDCS, and both should be more effective than the unihemispheric montage. Given the bilateral nature of the predicted plastic changes, the tDCS-related advantages should generalize to the untrained hand. Finally, bihemispheric tDCS should lead to activity changes in both hemispheres in a polarity-unspecific manner.

**Materials and methods**

**Participants**
Sixty-four healthy right-handed subjects (54.69% females; average age 22.84 ± 0.56 years) participated in this study. Participants completed the Edinburgh Handedness Inventory (Oldfield, 1971), as well as a survey of medical history, musical and computer gaming experience, and previous exposure to brain stimulation. Exclusion criteria for participation were as detailed previously (Waters-Metenier et al., 2014). Participants gave written informed consent in accordance to the Declaration of Helsinki, and received financial remuneration with the possibility of additional bonuses for completing the study and performance improvements. The protocol was approved by the UCL Research Ethics Committee.

**Sequence task**
The experiment required the fast production of different unimanual 5-digit sequences. The sequences were performed on a custom-built, MRI-compatible, piano-like isometric force
keyboard. Forces exerted on each key were measured every 5 ms via transducers (Sensing &
Control Honeywell Inc., PSG-15N1A, dynamic range of 0-25 N).

The sequence task required participants to press each digit in a pre-defined order. A
computer screen showed each sequence as a specific ordering of the numbers 1-5. ‘1’ referred to
left-most and ‘5’ to right-most key, and the sequence was executed from left to right. In other
words, sequences were cued in an extrinsic, spatial reference frame. Based on pilot
experimentation (Wiestler and Diedrichsen, 2013), we selected twelve sequences of matched
difficulty, excluding sequences that contained a run of more than three adjacent digits. From
these twelve sequences, each participant was assigned a set of four sequences that would be
trained (Wiestler et al., 2014). The possible sets for training were (41352, 25314, 15423,
51243), (45132, 21534, 31425, 35241), and (52134, 14532, 23541, 43125).

A small (0.53 cm x 0.53 cm) green box flanking the presented sequence was displayed on
the left and right to cue the respective hand that should execute the sequence. A red box
appeared on the other side indicating that the hand should remain at rest on the keyboard. The
instructional stimulus remained on the screen for 2.7 s, after which five white asterisks were
presented as a ‘go’ signal. Each instructed sequence was executed either four times in a row (in
the pre-test and post-test) or three times (during training and fMRI acquisition), and each
execution was individually triggered with a ‘go’ signal. There was a 500 ms interval between
consecutive sequence executions. For the remainder of the manuscript, we define a single ‘trial’
as the set of three or four consecutive executions of the same sequence.

The objective of the sequence task was to perform the five digit presses as quickly as
possible with minimal errors. For a digit press to be registered, the active digit had to exceed
exerting a force of 2.5 N, while all other digits had to generate forces of below 2.2 N. After each
digit press, the corresponding asterisk changed color to provide feedback about whether the
individual press was: ‘correct’ (green), ‘incorrect’ (red), or ‘too hard’ (yellow; that is, greater
than the upper limit set for the task, 8.9 N). Execution time (ET) was measured as the duration
between onset of the first press and release of the last press, and error rate was defined as the
percentage of sequences that contained one or more incorrect digit presses. Throughout
behavioral training, a constant error rate was encouraged by instructing participants to speed
up if error rate was lower than 20% and slow down if it was higher.

After each sequence execution, participants saw a brief feedback (0.8 s): one green
asterisk (= 1 point) indicated that all five presses of the sequence were executed correctly; three
green asterisks (= 3 points) meant that the sequence was executed correctly and with ± 20%
faster ET than the average of the previous run; one blue asterisk specified that the sequence was
performed 20% slower than the average of the previous run (= 0 points); and one red asterisk
signified that one or more errors were made (−1 point). Participants received a financial bonus according to their point score.

**Experimental Design**

All participants completed 4 study phases (Fig. 1): a pre-test (day 1) in which baseline performance for twelve 5-digit sequences was evaluated for both hands; a 4-day training phase (days 2-5) during which participants repeatedly practiced the same four sequences with either the left or right hand for approximately an hour (which was coupled with 25 min of tDCS from the onset of training); a post-test (day 6), which was conducted in the same manner as the pre-test; and an MRI session (day 7).

The pre-test started with a short practice run with 4 trials or 16 executions (4 executions of two easy sequences per hand) to familiarize participants with the task. During the pre-test, participants performed all 12 sequences (4 to-be-trained and 8 untrained) with both left and right hands. Each hand was required to perform 2 trials per sequence with 4 executions per trial (i.e. 8 total executions). The pre-test consisted of eight runs with 24 trials per hand, which resulted in a total of 96 executions per hand. Within the first four runs, the order of sequences and hands was randomly permuted, and the order was reversed in the second half to counterbalance possible learning effects.

We assigned subjects pseudo-randomly to one of four stimulation groups (sham, unihemispheric, bihemispheric, or reversed-polarity bihemispheric tDCS groups) and to training cohort (left or right hand training), such that group differences in pre-test performance were minimized (Waters-Metenier et al., 2014). ANOVA across tDCS groups revealed no difference in baseline task ET ($F_{(3,30)} = 0.158, p = 0.923$) (see Table 2 for individual group means), and there was also no significant pair-wise difference between any two stimulation groups (all $t < 1.10, p = 0.273$).

At the beginning of each session, participants provided information about sleep quality, alertness, attention, and task difficulty using visual analogue scales ranging from 0 (lowest) to 10 (highest). There were no significant differences between stimulation groups in terms of these parameters (Table 2).

To ensure that both participants and experimenter were blind to tDCS assignment, the randomisation was performed by a JD, who was not involved in data collection. The experimenter (SW) only knew the hand training cohort and the electrode arrangement, but not whether the participant received real or sham stimulation, which was determined by a randomized code entered into the tDCS machine at the beginning of each session. Accordingly, 66.7% of the sham participants (14/21) had bihemispheric electrode arrangement and 33.3% had unihemispheric electrode arrangement. No significant behavioral differences were found
between these two subsets of the sham group on post-test performance \( (F_{1,60} = 1.454, \ p > 0.245) \), ANCOVA using pre-test as covariate for trained/untrained hand and trained/untrained sequence ET.

During the four training days, participants practiced 4 of the 12 sequences with either their left or right hand. A session consisted of 16 runs with 2 trials per sequence each. Therefore, participants performed 128 trials (384 sequence executions) per day. On the day following the final session of tDSC-coupled training, a post-test that had exactly the same structure as the pre-test was administered.

**Transcranial direct current stimulation**

tDCS was administered via a bihemispheric or unihemispheric montage. In the unihemispheric montage, we placed the anode over contralateral M1 and cathode over ipsilateral supraorbital ridge. For the bihemispheric montage, we positioned the anode over contralateral and the cathode above ipsilateral M1, with the reversed-polarity montage involving the opposite polarity. The hand area of M1 was localized as the position where single-pulse suprathreshold TMS evoked a visible twitch in the contralateral first dorsal interosseus muscle (Boroojerdi et al., 1999). This was implemented using a Magstim Bistim2 with a 5-cm figure-of-eight coil positioned tangentially to the medial-sagittal plane skull at a 45° angle and with the handle pointing posteriorly and a monophasic pulse was delivered. This angle was chosen as it elicits the strongest the strongest perpendicular fields (Janssen et al., 2015) which is optimal for stimulating corticospinal neurons trans-synaptically via horizontal cortico-cortical connections (Di Lazzaro et al., 2008; Delvenne et al., 2014). tDCS was administered over four consecutive training days for the first 25 min of an approximately 60-min session of sequence training. A current of 2 mA was delivered using a neuroConn DC-stimulator PLUS (http://www.neuroconn.de/dc-stimulator_plus_en/) through saline-soaked 35 cm² electrodes.

**Behavioral analysis**

There was no significant difference between the tDCS groups in terms of error rate during the pre-test \( (F_{1,60} = 0.252, \ p = 0.860) \); averaged across training group and hand) or training \( (F_{1,60} = 1.082, \ p = 0.364) \); averaged across day). We only found a significant effect of tDSC on error rate in the post-test, with tDCS recipients tending to be more accurate than sham \( (F_{1,60} = 3.086, \ p = 0.0339) \); averaged across training group and hand).

To adjust ET for the different error rates, we calculated the median ET for each run, sequence, and hand over all (correct and incorrect) trials. For this calculation, ET for the incorrect trial was replaced with the maximum ET of that group of trials, thereby penalizing inaccurate performance.
We conducted pre-planned statistical comparisons between each stimulation group and sham, between bihemispheric and unihemispheric groups, and between the two bihemispheric groups for the last training day and the post-test. For these comparisons between pairs of groups, we used an ANCOVA in which the error-adjusted ET at pre-test was used as a covariate to account for prior inter-individual differences in performance. This procedure effectively subtracts from each training/post-test measurement the best prediction based on the pre-test measurement. Compared to simply subtracting the pre-test measurement, this method is less susceptible to noise induced by the larger variability in the pre-test measurements. The ANCOVA included 'tDCS group' and 'hand training cohort' as between-subject factors. The threshold for all statistical comparisons was $p < 0.05$. All data presented in the text and figures are represented as mean ± standard error of the mean (SEM).

**fMRI data acquisition**

All 42 bihemispheric tDCS recipients (sham and real) underwent fMRI scanning one day after the post-test. Due to resource limitations, we were not able to also scan the unihemispheric groups. While this group would have supplied additional information, it was not critical to test our main hypothesis of polarity-independent changes in both hemispheres after bihemispheric stimulation. At this point, at least 48h had elapsed since the final tDCS application. Therefore any group differences should be due to long-term neuroplastic changes induced by tDCS; immediate online effects of tDCS (Vernieri et al., 2010; Mielke et al., 2013; Antal et al., 2014) should have been washed out. The unihemispheric tDCS recipients were not scanned.

The fMRI session consisted of 8 runs comprised of 24 randomly ordered trials (3 per trial type—4 sequences x 2 hands—with 3 sequence executions per trial, yielding 72 total executions per run). Each trial consisted of a cueing phase (2.7 s, 1 TR), followed by 3 executions of the cued sequence, triggered 3.6s apart. A trial, therefore, lasted 13.5s (5 TRs). Each sequence execution had to be completed within 2.8s to allow for a 0.8s feedback phase.

To match behavioral performance during scanning, participants were instructed to produce the sequence at a fixed speed of 1.3s and as accurately as possible. This ET was the fastest that subjects across all groups could achieve with both trained and untrained hands with high accuracy (~90% correct). Additionally, throughout training, force levels of the sequences were kept similar by imposing a maximal force threshold. No force level feedback was provided during fMRI scanning.

Baseline BOLD activation was measured during 8 randomly interspersed rest phases of 13.5s. To monitor for mirror activity on the non-moving hand, participants were required to keep all ten digits on the keyboard and to generate a small baseline force of ~0.5N at all times.
Functional images were acquired using a 3T Siemens Trio MRI machine, with a 32-channel head coil. A 2D echo-planar sequence with a TR of 2.72s was used to acquire the functional volumes (8 runs, 159 volumes per run, 32 interleaved slices with 2.7 mm thickness) in an interleaved manner (3mm gap, and 2.3x2.3 mm² in-plane resolution). The volumes were acquired in an oblique orientation with a 45° tilt angle from the AC-PC line—this slice prescription provided coverage of motor regions on the dorsal surface of the cortex, as well as the superior cerebellum and basal ganglia, but excluded inferior prefrontal and inferior and anterior temporal lobes. For full details of the fMRI acquisition, see (Wiestler et al., 2014).

fMRI analysis

The analysis of the fMRI data is described in detail in Wiestler et al. (2014), which reports the results from the sham group. After standard pre-processing (correction for slice acquisition, motion realignment, and coregistration to the individual anatomical image), we used a first-level linear model implemented in SPM8 (Friston et al., 2007) to estimate the activation for each of the four sequences for each hand. The design matrix consisted of a regressor for each hand and sequence type and an intercept for each run. The regressor was modelled as a boxcar function of 10.8s beginning with the first go cue of the trial, which was then convolved with a standard hemodynamic response function. From the estimated regression weights, we computed the percent signal change compared to rest for each hand, averaged across the four sequences.

For the group analysis, we reconstructed the cortical surface of each individual participant using Freesurfer (Dale et al., 1999), which permits the extraction of the white-grey matter surface and pial surface from anatomical images. The functional data was then projected onto each individual surface by averaging for each surface node the voxels that lay between the white-grey matter and pial surfaces.

The individual surfaces were then aligned to a shared spherical template for left and right hemispheres. This allowed us to flip the results for the right hand trained cohort, such that data from the trained hemisphere—the hemisphere contralateral to the trained hand—was displayed on the right group hemisphere, and the data from the untrained hemisphere on the left. We then conducted t-tests between sham and tDCS groups for each surface vertex, using an uncorrected height threshold of $T_{139} = 2.71, p = 0.005$. Family-wise error was controlled by calculating the critical size of the largest suprathreshold cluster that would be expected by chance, using Gaussian Field theory as implemented in the fmristat package (Worsley et al., 1996). Results were displayed using the 3D-visualisation software Caret (Van Essen et al., 2001).
For the profile plots shown in Fig. 3a,b, we defined a line running from the posterior parietal cortex through the hand area of M1 to the anterior tip of Brodman area 6 (premotor cortex). We then averaged activity over the area 1.5 cm above and below that line (purple area in Fig. 3d).

We also conducted a region of interest (ROI) analysis for the cortical motor regions. They included the hand region of primary motor cortex (M1, Brodman area 4); primary somatosensory cortex (S1, Brodman area 1-3); dorsal premotor cortex (PMd); supplementary motor areas (SMA/pre-SMA); and superior parietal lobe, divided into an anterior (intra-parietal sulcus, IPS) and posterior (occipito-parietal junction, OPJ) aspect. We defined all ROIs on the symmetric group template and subsequently projected this into individual data space via the respective individual surface.

The average activity in these ROIs was submitted to an ANOVA to calculate differences between tDCS groups (factor 'tDCS') and interactions with training hand (factor 'hand') and hemisphere (factor 'hemisphere'). Because there were no significant functional differences between left and right hand training cohorts ($F_{(1,36)} = 2.21$, $p > 0.15$, for all 6 ROIs, even without correction), or interactions with any other factor, we analyzed the functional data averaged over left and right hand training cohorts.

**Results**

**Bihemispheric tDCS increases motor learning more than unihemispheric tDCS**

We first determined whether conventional bihemispheric tDCS is more effective in promoting learning than unihemispheric stimulation. We used the error-adjusted execution time (ET, see methods) as an overall performance measure. By the end of 4 days of sequence training, unihemispheric tDCS recipients executed sequences 0.203 ± 0.101 s (16.6%) faster than sham ($F_{(1,31)} = 4.43$, $p = 0.043$; Fig. 2a/b, black vs. blue lines). As in previous reports (Reis and Fritsch, 2011), this advantage persisted during the post-test, which was conducted without tDCS one day after the final training session ($F_{(1,31)} = 8.13$, $p = 0.008$). There was no significant difference between the left and right hand trained groups in the size of this effect ($F_{(1,31)} = 2.03$, $p = 0.164$).

Participants who received conventional bihemispheric tDCS were an additional 0.260 ± 0.114s faster than those who received unihemispheric stimulation. The difference between bihemispheric and unihemispheric groups was reliable ($F_{(1,24)} = 12.44$, $p = 0.002$), as was the difference between bihemispheric and sham groups ($F_{(1,30)} = 64.02$, $p = 6.24\times10^{-9}$). These differences were maintained during the post-test (respectively, $F_{(1,24)} = 10.98$, $p = 0.003$ and $F_{(1,30)} = 55.302$, $p = 2.75\times10^{-9}$). Hence, moving the cathode from the supraorbital ridge (unihemispheric tDCS) to ipsilateral M1 (bihemispheric tDCS) yielded nearly twice the
performance gain (37.8% relative to sham). Therefore, we replicate here higher effectiveness
for bihemispheric tDCS relative to unihemispheric stimulation (Vines et al., 2008; Mahmoudi et
al., 2011; Karok and Witney, 2013; Lindenberg et al., 2013; Sehm et al., 2013; Naros et al., 2016)
in the context of a multiple-day learning study.

Reversed-polarity bihemispheric tDCS increases motor learning

The interhemispheric competition model supposes that the advantage of bihemispheric relative
to unihemispheric tDCS arises due to cathodal suppression of ipsilateral cortex. This idea
predicts that the reversed-polarity montage should attenuate motor learning relative to sham,
as it decreases contralateral M1 excitability both directly through cathodal and indirectly
through ipsilateral anodal stimulation.

Our data, however, showed the converse: reversed-polarity bihemispheric tDCS led to
significantly faster ETs than sham stimulation, both during the final training day \( F_{(1,33)} = 27.55,\)
\( p = 1.154 \times 10^{-6} \) and during the post-test \( F_{(1,30)} = 25.0, p = 2.32 \times 10^{-6} \). Statistically, the performance
of the reversed polarity group was indistinguishable from conventional bihemispheric tDCS
(last training day: \( F_{(1,23)} = 1.81, p = 0.191 \), post-test: \( F_{(1,23)} = 1.93, p = 0.178 \)). The ETs for the
conventional and reversed-polarity bihemispheric groups were also symmetric across left and
right hand trained groups: the tDCS group \( x \) hand cohort interaction was not significant \( F_{(1,23)} \)
\( =0.11, p = 0.7459 \). Even though reverse-polarity bihemispheric tDCS led numerically to better
outcomes than unihemispheric tDCS, this effect did not reach statistical significance (last
training day: \( F_{(1,24)} = 2.97, p = 0.097 \), post-test: \( F_{(1,24)} = 2.02, p = 0.168 \)). However, the two
bihemispheric groups combined were significantly faster than the unihemispheric group (last
training day: \( F_{(1,38)} = 8.61, p = 0.006 \), post-test: \( F_{(1,24)} = 6.66, p = 0.014 \)).

Collectively, these results indicate that bihemispheric stimulation was more effective
than unihemispheric stimulation. This additional benefit, however, was not conferred by
ipsilateral suppression, as we did not find a polarity-specific effect for bihemispheric tDCS.
Instead, any stimulation of the ipsilateral motor areas accelerated motor learning.

These results, therefore, favor the bihemispheric cooperation model, by which the
additional learning advantage of bihemispheric relative to unihemispheric tDCS arises from
plastic changes in both hemispheres that would promote performance for both hands. This idea
makes two testable predictions. First, a considerable part of the behavioral tDCS advantage
should generalize to the untrained hand. Secondly, neural changes should occur in both
hemispheres in a polarity-unspecific manner. In the remainder of the paper, we test these
predictions.
Behavioral tDCS effects generalize to the untrained hand

In the pre-test and post-test, participants This generalization was even more pronounced in the tDCS groups: relative to sham, unihemispheric performed trained sequences with their untrained hand, allowing us to assess inter-manual generalization. Consistent with previous results (Waters-Metenier et al., 2014; Wiestler et al., 2014), even the sham group showed considerable performance improvements on the untrained hand (Fig.2c/d). \( F_{(1,31)} = 12.43, p = 0.001 \), bihemispheric \( F_{(1,23)} = 26.99, p = 1.34e^{-09} \), and reversed-polarity bihemispheric groups \( F_{(1,24)} = 25.98, p = 1.78e^{-09} \) all performed significantly better on the untrained hand. Additionally, performance for the untrained hand was better in bihemispheric relative to unihemispheric tDCS recipients \( F_{(1,31)} = 4.26, p = 0.047 \), and there was no difference between the bihemispheric tDCS groups \( F_{(1,23)} = 0.05, p = 0.822 \).

Importantly, the enhancement of untrained hand performance was even larger than what would have been expected if generalization were simply proportional to the improvements on the trained hand: when expressing pre-/post-test difference for the untrained hand relative the learning gains for the trained hand, we found that tDCS increased the proportion of generalization. For sham recipients, the untrained hand gained 58.5% of the improvement of the trained hand, whereas, for both bihemispheric tDCS groups, the percentage of inter-manual generalization was greater (86-89.2%; \( t_{(1,33)} > 2.662 \), \( p < 0.012 \)). These results suggest that bihemispheric tDCS influenced mainly effector-independent representations in both hemispheres.

Behavioural tDCS effects generalize to untrained sequences

All participants also improved on the untrained sequence from pre-test to post-test (see Fig. 2d). This effect was promoted by tDCS, such that, for untrained sequences, unihemispheric tDCS recipients were 0.310 ± 0.105 s (19.4%) faster than sham and bihemispheric recipients (conventional and reversed-polarity groups combined) were 0.231 ± 0.137 s (17.6%) faster than unihemispheric anodal tDCS recipients (and both of these differences were significant; respectively \( F_{(1,31)} = 6.73, p = 0.014 \) and \( F_{(1,36)} = 8.13, p = 0.008 \)). Additionally, when we quantified the proportion of transfer of speed advantages to untrained sequences for the trained hand, we found that, across all groups, tDCS increased the proportion of transfer relative to sham \( F_{(3,30)} = 4.90, p = 0.0041 \). Therefore, the effect of tDCS in this study is exerted at a largely effector- and sequence-unspecific fashion, as we have reported previously (Waters-Metenier et al., 2014).
Bihemispheric tDCS causes activation increases in both hemispheres

To elucidate the neural consequences of tDCS stimulation, we measured fMRI BOLD activation while participants executed the four sequences with either the trained or untrained hand. Participants were scanned two days after their final tDCS-coupled training session, such that our measure would reflect learning-related plasticity, rather than immediate effects or aftereffects of tDCS on neural excitability or hemodynamics which might not be directly related to sequence learning (Vernieri et al., 2010; Mielke et al., 2013; Antal et al., 2014). To prevent activity differences attributable to simple behavioural differences, we closely matched the performance in the scanner in terms of movement speed and force across both groups and hands. The hemispheric cooperation model predicts that similar neural changes should occur in both hemispheres, independently of polarity. As expected, for trained hand executions, the sham group exhibited contralateral activation and ipsilateral deactivation in M1 and S1 (Fig. 3a, Fig. 4), as often observed during simple unimanual hand movements such as those that we study here (Diedrichsen et al., 2013; Westler and Diedrichsen, 2013). In contrast, both bihemispheric tDCS groups—independently of polarity—showed greater contralateral activation and no ipsilateral deactivation.

For statistical comparison, we combined the two bihemispheric stimulation groups into a single 'tDCS' group, as we did not find any significant clusters of differential activation between conventional and reversed-polarity tDCS groups. A surface-based group analysis (see Materials and methods) showed that both contralateral and ipsilateral primary somatosensory (S1) and primary motor (M1) cortex had greater activation in tDCS recipients (Table 3) for movements of the trained hand. Functional differences for the untrained hand were visually similar, but statistically less pronounced (Fig. 3b; Fig. 4b; Table 3).

Analysis using anatomically predefined regions of interests (ROIs) (Westler and Diedrichsen, 2013), confirmed these results (Fig. 3e). Across both hemispheres and hands, we observed activation increases in M1 ($F_{(1,40)} = 9.34$, $p = 0.004$) and S1 ($F_{(1,40)} = 13.55$, $p = 0.0007$). There was some evidence for an interaction of tDCS x hemisphere x hand in S1 ($F_{(1,40)} = 4.16$, $p = 0.048$), although this did not quite reach significance in M1: $F_{(1,40)} = 3.72$, $p = 0.061$. This reflects that bihemispheric tDCS especially increased activation associated with the trained hand and ipsilateral (untrained) hemisphere.

We also observed the expected hemispheric asymmetries with more ipsilateral activity in the left hemisphere during left-hand movements as compared to activity in the right hemisphere during right-hand movements (Verstynen et al., 2005). This hemispheric asymmetry was significant for M1 ($F_{(1,39)}=31.137$, $p=1.983e^{-6}$) and S1 ($F_{(1,39)}=21.73$, $p=3.621e^{-5}$), but did not interact significantly with the tDCS effect.
Despite our best efforts to match behavioral performance during fMRI, there were slight, but significant differences between the groups (Table 4). However, the differences in ET were very small (on the order of 40-60 ms), and the differences in force relative to sham were not consistent across the two bihemispheric tDCS groups (force was slightly lower than sham in the conventional bihemispheric tDCS group and slightly higher than sham in the reversed-polarity tDCS group). Nevertheless, to ensure that the observed increases in activation could not be attributable to these small behavioral differences, we included execution time, error rate, and force as covariates in ANCOVA analyses and found that, for all comparisons, the effect of tDCS remained significant for both M1 (respectively, $F_{1,39} = 7.26$, $p = 0.01$; $F_{1,39} = 14.03$, $p = 0.001$; $F_{1,39} = 8.29$, $p = 0.006$) and S1 ($F_{1,39} = 8.02$, $p = 0.007$; $F_{1,39} = 10.99$, $p = 0.002$; $F_{1,39} = 12.78$, $p = 0.001$).

Another potential confound that could lead to increased ipsilateral activation is mirroring. Mirroring refers to the phenomenon whereby muscles of the non-moving hand are activated simultaneously with those of the moving hand (Beaule et al., 2013). Such movements are typically visible in pathological states (e.g., stroke), but are also present and measurable in healthy populations. During fMRI, participants were required to rest the passive hand on the keyboard while the active hand was executing sequences. Mirroring was parameterized using the range of forces on the passive hand across the time course of a trial, the associated standard deviation, and the correlation between the force traces for matching digits of the passive and active hand. Even though the significant positive correlations indicated that we could successfully detect the very subtle mirroring in our healthy control participants, there were no significant difference between tDCS groups for any of these measures (Table 5).

Taken together, these data demonstrate that bihemispheric tDCS—irrespective of the polarity—was associated with increases in average activation in both ipsilateral and contralateral hemispheres. This difference could not be explained by behavioral differences between the groups during the scan.

**Quantification of subject blindedness and perceptual side effects of tDCS**

Subsequent to each session of tDCS administration, participants underwent a previously designed battery to characterize tDCS effects (Waters-Metenier et al., 2014), with the addition of questions about perceptual side effects. Specifically, participants were asked to rate the experience of tingling, pain, burning, itching, dizziness, and mental fatigue (1:10) and then to describe (in min) the duration of the effect. As can be seen in Fig. 5a, tDCS recipients tended to exhibit slightly higher intensities of perceptual properties of tDCS—especially in terms of tingling, burning and itching—however, none of these variables showed a significant between-
group difference (all \( t_{(62)} < 1.446, p > 0.153 \)) or exceeded level 4 (out of a maximal rating of 10) for any of the 64 subjects.

However, the groups tended to differ on how long they experienced these side effects (Fig. 5b). Averaged across the three tDCS groups (unihemispheric, bihemispheric and reversed-polarity bihemispheric), subjects reported significantly longer durations of tingling \( (t_{(62)} = 2.553, p = 0.013) \), burning \( (t_{(62)} = 2.492, p = 0.015) \), and itching \( (t_{(62)} = 2.833, p = 0.006) \), and a tendency for longer duration of pain \( (t_{(62)} = 1.806, p = 0.076) \). There were no differences in the duration of dizziness \( (t_{(62)} = 1.051, p = 0.298) \) or mental fatigue \( (t_{(62)} = 0.671, p = 0.505) \) between sham and tDCS groups. As observed previously (Waters-Metenier et al., 2014), participants experienced no significant differences in overall discomfort, perceived tDCS intensity, or distraction due to tDCS (Table 2). Moreover, \( \chi^2 \) goodness of fit tests showed that there were no differences in the detectability of tDCS assignment between tDCS groups and sham (Table 2). These findings collectively suggest that, despite slight perceptual differences, tDCS blinding methods were sufficient. This is congruent with other recent evidence (Kessler et al., 2012), including a study that investigated over twice as many subjects at 2 mA (Russo et al., 2013).

Assessment of behavioral side effects of tDCS

In addition to the main training task (the sequence task described above), we tested all bihemispheric tDCS and sham recipients on two additional tasks of manual skill during the pre-test and post-test: individuation (the ability to move the digits separately) and configuration execution (the skill of pressing certain digits at the same time while keeping coactivation of unintended digits minimal)—for full details of these tasks, see Waters-Metenier et al. (2014).

Neither bihemispheric tDCS group exhibited any side effects from tDCS-coupled sequence training on either of these skills (Fig. 5c, d), and there were no significant differences between the two bihemispheric groups and sham (all \( F_{(2,35)} < 2.092, p > 0.139 \); using ANCOVA correction with pre-test performance). Therefore, as in our previous work, we did not find any adverse trade-offs between using tDCS to facilitate manual motor skill learning and performance on untrained tasks (Waters-Metenier et al., 2014).

Discussion

Our study provides evidence for an active role of ipsilateral motor regions in unimanual motor skill learning. We replicated the classic observation that bihemispheric tDCS is more effective than stimulating only one hemisphere (Vines et al., 2008). Critically, bihemispheric tDCS with the anode over ipsilateral motor cortex led to similar learning advantages as with the anode
over contralateral motor cortex. Finally, both montages led to long-lasting increases of functional activity in bilateral sensorimotor areas and the tDCS-induced learning advantage generalized to the untrained hand.

Our results clearly argue against the interhemispheric-competition model as an explanation for the advantage of bihemispheric over unihemispheric tDCS. According to this idea, cathodal stimulation suppresses the ipsilateral hemisphere, subsequently releasing the contralateral hemisphere from interhemispheric suppression. Our results for the reversed-polarity bihemispheric group are at odds with this explanation, because excitatory stimulation of the ipsilateral cortex should have led to attenuated, rather than accelerated, motor learning relative to unihemispheric (or even sham) stimulation.

Instead, the full pattern of our results can be explained under the assumption that tDCS increased plasticity in both hemispheres independently of polarity, and that the two motor cortices cooperate in producing high levels of skill (see Table 1). One limitation of our study is that we did not directly measure changes in bilateral MEPs before and after tDCS. Thus, we cannot make strong inference about whether the cathodal stimulation in the bihemispheric montage increased or decreased short term excitability in M1. For example, there is some evidence that cathodal unihemispheric tDCS stimulation at 2 mA increases, rather than decreases, MEPs (Mordillo-Mateos et al., 2012; Batsikadze et al., 2013; but see, Cengiz et al., 2013). Importantly, previous studies of the effect of bihemispheric tDCS on MEPs have not exhibited consistent excitability changes using a bihemispheric montage: in contrast to unihemispheric tDCS, bihemispheric stimulation has been found to either produce no significant changes in MEPs (O’Shea et al., 2014), or changes that were statistically less robust (Mordillo-Mateos et al., 2012). These results raise the possibility that the changes in motor plasticity shown behaviorally in this and previous (Vines et al., 2008) studies, rely on different mechanisms than those reflected in the polarity-specific changes in MEPs. A parsimonious explanation for our results is that the behavioral tDCS effects are related to the spatial distribution of electrical currents, rather than to the current direction. Biophysical current modelling of tDCS (Truong et al., 2014; Naros et al., 2016) demonstrates that the unihemispheric montage primarily sends current through contralateral premotor and ipsilateral prefrontal regions, whereas bihemispheric stimulation targets motor and premotor regions bilaterally. Importantly, the weak radial currents that give rise to the polarity-specific effects on MEPs switch directions on opposite sides of the gyrus (Rahman et al., 2013). Therefore, premotor areas in both hemispheres could experience either suppression or excitation depending on their folding geometry. The effects of tDCS on neuroplasticity, therefore, could be mediated by the considerably stronger tangential currents, which, in contrast to the radial currents, do not have polarity-specific effects (Rahman et al., 2013). Under
this assumption, bihemispheric tDCS (independently of polarity) would have increased neural
plasticity in both hemispheres in a manner unrelated to the changes measureable with MEPs.

Even under the assumption of polarity-unspecific tDCS effects on plasticity, our results
remain incompatible with the hemispheric competition model: if the cathode promoted
plasticity in ipsilateral M1 during training, then the interhemispheric competition model would
have predicted a disadvantage of both bihemispheric montages relative to the unihemispheric
montage, as bihemispheric stimulation would increase the putatively harmful ipsilateral
activation (see Table 1).

Instead of probing excitability of the primary motor cortex after tDCS stimulation using
tMRS, we evaluated task-related activity using fMRI after transient tDCS effects had been washed
out. An advantage of this approach is that we avoided possible interference with the process of
memory consolidation through the necessary TMS stimulation to M1 when measuring MEPs
(Muellbacher et al., 2002). We observed that the average activity during trained hand
movements was larger in bihemispheric tDCS groups in both contralateral and ipsilateral
sensorimotor regions. The results for the untrained hand were similar, albeit less robust.
Previous work has demonstrated bilateral activity increases in M1 during the application of
bihemispheric tDCS (Lindenberg et al., 2013). Similar online (Kwon and Jang, 2011; Stagg et al.,
2012) or short-lasting (Baudewig et al., 2001; Kwon et al., 2008; Jang et al., 2009; Stagg et al.,
2009; Kim et al., 2012) changes underneath the anode have also been reported for
unihemispheric tDCS. Importantly, we measured functional activity approximately 48 hours
after the end of the final stimulation. Therefore, the activity increases reported here reflect
longer-lasting changes caused by neural plasticity, rather than any immediate effects of tDCS on
the hemodynamic response.

The fact that the activation changes in our study were restricted to primary motor and
sensory cortex should not necessarily be taken as evidence that the relevant neuroplastic
changes only occurred here. Rather, it is equally possible that tDCS led to increased plasticity
bilaterally in premotor or supplementary motor cortex, and that the increased activity in M1
and S1 reflects the increased modulatory input from these areas.

Increased plasticity in motor and pre-motor areas of both hemispheres would also
explain our observation that the behavioral advantages due to tDCS generalized to the
untrained hand. In a previous study, we used multi-voxel pattern analysis to identify
commonalities in the neural encoding of specific motor sequences for the left and right hands
(Wiestler et al., 2014). We found widespread, shared representations in both premotor and,
surprisingly, primary motor areas of both hemispheres. Given that tDCS increased the amount
of intermanual generalization, it appears likely that effector-independent sequence
representations were particularly facilitated.
To summarize, we demonstrate that conventional and reversed-polarity bihemispheric tDCS similarly increase motor learning and BOLD activation in both anode- and cathode-modulated hemispheres. Thus, our study provides evidence for an active role of the ipsilateral cortex in unimanual motor control in young, healthy individuals, consistent with previous reports in older adults or victims of stroke (Johansen-Berg et al., 2002; Zimerman et al., 2014). tDCS effects on plasticity are still often construed in terms of excitation/inhibition of neural tissue under the electrodes, and the interhemispheric competition model is commonly used to explain the superiority of bihemispheric tDCS. The idea of promoting motor learning by reducing ipsilateral excitability is particularly pertinent to stroke neurorehabilitation, where it has led to the supposition that suppressing activity in the healthy hemisphere to release inhibition of lesioned cortex may facilitate recovery (Hummel and Cohen, 2006; Williams et al., 2010; Bolognini et al., 2011; Nitsche and Paulus, 2011; Takeuchi and Izumi, 2012; Zimerman et al., 2012; Krause et al., 2013; Lefebvre et al., 2014). While the hemispheric cooperation model proposed here must be tested in elderly participants and individuals with stroke, our data suggest that a simple excitation/inhibition model may be too simplistic and should be abandoned in favor of a framework that acknowledges the broad effects of tDCS current and the roles that both hemispheres play in the encoding of information during motor learning.

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Figure captions

Figure 1: Experimental Design.
(a) tDCS montages: sham stimulation (blue); unihemispheric (‘Uni’) tDCS (black); bihemispheric (‘Bi’) tDCS (red); and reversed-polarity bihemispheric (‘Bi-RP’) tDCS (green). The location of the anode is indicated by a ‘+’ and the location of the cathode by a ‘−’. Here, the electrode placement for the left-hand trained group is shown (for which the right hemisphere was the ‘trained hemisphere’). For right-hand trained participants, the electrode location was reversed for all stimulation groups. For purposes of double-blinding, 2/3 of the sham group had a bihemispheric and 1/3 had an unihemispheric montage.

(b) Procedure: During the pre-test, participants performed 12 5-digit sequences with either hand. Subsequently, participants were assigned to one of the four tDCS groups and trained for 4 days with either the left or the right hand, resulting in 8 different groups (for details see Table 1). During the post-test, participants were tested again (as in the pre-test) without tDCS. Finally, all participants with a bihemispheric montage (but not with unihemispheric montage) underwent functional MRI scanning on day 7.

Figure 2: Bihemispheric tDCS accelerates learning and generalization in a polarity-independent manner.
(a) Average ET in the pre-test, training, and post-test for sham (blue), unihemispheric (black), bihemispheric (red), and reversed-polarity bihemispheric (green) tDCS groups. Subjects trained with either the left hand or (b) right hand. (c) Pre- and post-test data for the trained and (d) untrained sequences. Results are shown for the trained (T) and untrained (U) hands, separated by group. The pre-test results (grey dashed line) are averaged across all four groups. Error bars and shaded region indicate between-subject SEM.

Figure 3: Bihemispheric tDCS recipients show greater average activation than sham in bilateral sensorimotor areas.
(a) Profile plot of % signal change relative to rest in the trained and untrained hemispheres for trained hand executions. Results are shown for the sham, bihemispheric (Bi tDCS), and reversed-polarity tDCS groups (Bi-RP tDCS). The x-coordinate indicates the distance from the fundus of the central sulcus along the cortical surface in mm, running from occipital-parietal junction (posterior) to the rostral tip of premotor cortex (anterior). (b) Profile plot for the untrained hand. (c) Average sulcal depth along the profile. (d) Location of areas averaged in the profile plots on an inflated brain surface (purple area) (e) Average % signal change in 6
anatomically-defined ROIs (Wiestler and Diedrichsen, 2013; Wiestler et al., 2014). Brackets indicate that the two bihemispheric groups were significantly different from sham (**: p<0.0083, statistical threshold for multiple comparisons, *: p<0.05). Abbreviations: CS = central sulcus; IPS = intraparietal sulcus; PoSC = postcentral sulcus; SFS = superior frontal sulcus.

Figure 4: Bihemispheric tDCS recipients exhibit more activity in bilateral sensorimotor areas relative to sham.

(a) Activation maps for the trained and untrained hemispheres for the sham, bihemispheric (Bi tDCS), and reversed-polarity (Bi-RP tDCS) groups averaged across hand training cohort. While the sham group exhibited ipsilateral deactivation (typically found in fMRI studies of unilateral movements), this pattern was not found for either bihemispheric tDCS group. Additionally, tDCS groups exhibited higher contralateral activation. (b) Corresponding maps for sequences executed with the untrained hand. (c, d) Difference T-maps for the activation in the tDCS groups relative to sham for the trained (c) and untrained (d) hands.

Abbreviations: CS = central sulcus; IPS = intraparietal sulcus; PoSC = postcentral sulcus; SFS = superior frontal sulcus.

Figure 5: Online perceptual side effects of tDCS.

We monitored the potential online side effects of tDCS in order to evaluate perceptual differences between tDCS groups and sham. (a) For the intensity level of all six effect types, there were no significant differences across tDCS groups. (b) However, the differences between tDCS and sham tended to be greater for the duration of these side effects and significant differences were observed for duration of burning and itching (indicated by asterisks). (c) Post-test performance of the configuration task speed (left panel) and mean deviation (right panel) for the sham (blue), bihemispheric (red), and reversed-polarity bihemispheric (green) groups. (d) Post-test performance of individuation RMSE. tDCS recipients experienced no adverse behavioral effects as a result of tDCS-coupled sequence training.
1 Day Pre-test 12 Sequences

1 Day Post-test 12 Sequences

2 3 4 5

4 Sequences Training + tDCS (Sham, Uni, Bi, or Bi-RP tDCS)

or

0 min 25 min 60 min Training tDCS 2 mA tDCS

b

Pre-test
12 Sequences

Day 1

Training + tDCS (Sham, Uni, Bi, or Bi-RP tDCS)

or

Post-test

12 Sequences

Day 6

fMRI

Only bihemispheric

Untrained hemisphere

Trained hemisphere

a

Sham

Uni tDCS

Bi tDCS

Bi-RP tDCS
### Execution Time (s)

#### Left-hand trained

- **Pre-test**: 3.0
- **Training days 1-4**: Decreasing trend
- **Post-test**: 1.0

#### Right-hand trained

- **Pre-test**: 2.5
- **Training days 1-4**: Decreasing trend
- **Post-test**: 1.0

#### Trained sequences

- **Left-hand trained**: Decreasing trend
- **Right-hand trained**: Decreasing trend

#### Untrained sequences

- **Left-hand trained**: Decreasing trend
- **Right-hand trained**: Decreasing trend
a) Intensity and Duration of Sensations

- Tingling
- Pain
- Burning
- Itching
- Dizziness
- Fatigue

b) Configuration Task

- Execution Time (s)
- Mean Deviation
- RMSE

Left-hand trained
Right-hand trained

TU UT

Pre-test

Sham
Uni tDCS
Bi tDCS
Bi-RP tDCS

* Significant difference

C) Individuation Task

- Execution Time (s)
- Mean Deviation
- RMSE

Left-hand trained
Right-hand trained

TU UT

Pre-test
Table 1: Experimental prediction of hemispheric competition and cooperation models, assuming either polarity specific or polarity unspecific effects.

We have listed the conditions in the order of the predicted behavioral performance (< means lower movement time, i.e. better performance). The predictions for the hemispheric competition model assuming polarity-specific effects on plasticity and for the hemispheric cooperation model assuming polarity-unspecific effects are presented in the introduction. Under the hemispheric competition model with polarity-unspecific tDCS effects, stimulating contralateral M1 only would be expected to be more effective than stimulating both (since both M1s are competing). However, both bihemispheric montages should still be more effective than sham as contralateral M1 is being stimulated. Under the hemispheric cooperation model with polarity-specific tDCS effects, unihemispheric tDCS is expected to have the greatest facilitatory effect because contralateral M1 is being stimulated and ipsilateral M1 is unaffected. The exact prediction for the remaining three condition depends on the relative importance for each hemisphere in developing the skill.

| Polarity specific | Hemispheric competition model | Hemispheric cooperation model |
|-------------------|-------------------------------|-----------------------------|
| anodal increases, cathodal decreases plasticity | Bi < Uni < Sham < Bi-RP | Uni < Bi ≤ Bi-RP ≤ Sham |
| Polarity unspecific | Uni < Bi = Bi-RP < Sham | Bi = Bi-RP < Uni < Sham |
| both anodal and cathodal increase plasticity | | |
### Table 2: Participant demographic and psychological variables

To evaluate group differences in sex and detectability of tDCS status, all three tDCS groups were individually compared to sham using a χ² goodness of fit test. For all other parameters, mixed-effects ANOVA was calculated with the between factor ‘tDCS’. All variables were averaged across left and right hand training cohorts.

| Group | Sham | Uni tDCS | Bi tDCS | Bi-RP tDCS | Over all 4 groups |
|-------|------|----------|---------|------------|------------------|
| N (LH-trained; RH-trained) | 21 (11:10) | 15 (8:7) | 14 (7:7) | 14 (7:7) |                     |
| Sex (Female : Male) | 13 : 8 | 7 : 8 | 6 : 8 | 9 : 5 |                     |

| Detectability of tDCS status |  |
|------------------------------|---|
| tDCS status: (Yes: No) |  |
| 15 : 6 | 11 : 4 | 10 : 4 | 11 : 3 | 15.6 | 32.11 |

| tDCS perception |  |
|-----------------|---|
| Averaged across all training days |  |
| Discomfort (0:10) | 2.32 ± 0.46 | 2.72 ± 0.39 | 3.30 ± 0.54 | 2.88 ± 0.37 | 0.846 | 0.474 |
| Perceived Intensity (0:10) | 2.81 ± 0.40 | 3.28 ± 0.44 | 2.84 ± 0.62 | 3.91 ± 0.41 | 1.164 | 0.331 |
| Distraction due to tDCS (0:10) | 1.07 ± 0.31 | 1.80 ± 0.38 | 1.63 ± 0.43 | 1.57 ± 0.39 | 0.825 | 0.485 |

| Demographics |  |
|---------------|---|
| Age | 22.19 ± 0.95 | 24.00 ± 1.59 | 22.93 ± 0.99 | 22.43 ± 0.82 | 0.518 | 0.671 |
| Handedness (Edinburgh) | 88.09 ± 1.87 | 84.33 ± 4.57 | 77.5 ± 4.15 | 82.14 ± 3.95 | 1.661 | 0.185 |
| Previous motor training (hrs) | 640 ± 293 | 872 ± 396 | 447 ± 246 | 486 ± 306 | 0.336 | 0.800 |

| Baseline motor performance |  |
|---------------------------|---|
| Baseline ETA | 2.36 ± 0.065 | 2.33 ± 0.15 | 2.45 ± 0.10 | 2.32 ± 0.069 | 0.159 | 0.924 |

| Psychological measures |  |
|------------------------|---|
| Averaged across all days |  |
| Sleep Hours | 7.29 ± 0.21 | 6.94 ± 0.22 | 7.49 ± 0.19 | 7.49 ± 0.21 | 1.393 | 0.254 |
| Sleep Quality (0:10) | 7.28 ± 0.26 | 6.97 ± 0.19 | 7.89 ± 0.24 | 7.27 ± 0.26 | 2.155 | 0.102 |
| Alertness | 6.77 ± 0.35 | 6.62 ± 0.51 | 7.21 ± 0.55 | 7.12 ± 0.24 | 0.413 | 0.744 |
| Attention | 7.65 ± 0.28 | 7.56 ± 0.34 | 8.18 ± 0.32 | 7.04 ± 0.52 | 1.490 | 0.226 |
| Task Difficulty (0:10) | 4.56 ± 0.45 | 5.43 ± 0.42 | 4.71 ± 0.37 | 5.16 ± 0.30 | 0.993 | 0.403 |

| Averaged across final training day and post-test |  |
| Semantic Recall (% correct) | 73.21 ± 4.59 | 88.33 ± 3.33 | 96.43 ±1.57 | 93.75 ± 2.54 | 7.362 | 0.0003 |
Table 3: Effect of tDCS on average activation using surface-based analysis.

Between-subject analysis with uncorrected height threshold of $T_{(1,39)} = 2.71$, $p = 0.005$. Area indicates the size of the suprathreshold cluster, $T_{(1,39)}$ the maximal t-value, $P$-cluster is the corrected probability of observing a cluster of this size or bigger over the whole cortical surface by random chance (Worsley et al., 1996). Coordinates reflect the location of the cluster peak in MNI space.

| Region (Brodmann Area) | Area (mm²) | Peak value $T_{(1,39)}$ | $P$ (clust) | MNI coordinates |
|------------------------|------------|------------------------|-------------|-----------------|
| **AVERAGE ACTIVATION** |
| tDCS>sham (% signal activation > rest) |
| **Trained hand** |
| Contralateral (Trained) hemisphere |
| Postcentral (S1; BA3) | 145.33 | 3.64 | 0.007 | 49.89 | -22.22 | 51.78 |
| Postcentral (S1; BA3) | 116.21 | 3.81 | 0.030 | 18.26 | -39.58 | 65.61 |
| Precentral (M1; BA4) | 114.01 | 3.78 | 0.033 | 37.77 | -20.16 | 57.22 |
| Ipsilateral (Untrained) hemisphere |
| Precentral (M1; BA4) | 574.01 | 5.11 | <0.001 | -37.41 | -26.36 | 55.81 |
| Postcentral (S1; BA3) | 229.65 | 4.45 | <0.001 | -17.03 | -39.31 | 69.61 |
| Orbital area / Pars triangularis (BA47) | 182.63 | 5.49 | 0.002 | -42.46 | 29.98 | -0.78 |
| **Untrained hand** |
| Contralateral (Untrained) hemisphere |
| Orbital area / Pars triangularis (BA47) | 116.31 | 4.39 | 0.031 | -41.68 | 30.01 | -0.44 |
| **Ipsilateral (Trained) hemisphere** |
| All non-significant |
Table 4: Execution time, error rate, and force during fMRI.
Table shows mean (±standard error) of behavioral parameters for all three tDCS groups during fMRI scanning (averaged across hand training cohort). The last column indicates an F-test comparing the 3 groups.

|                | Sham mean | SE   | Bi-DCS mean | SE   | RP-Bi-DCS mean | SE   | ANOVA across groups |
|----------------|-----------|------|-------------|------|----------------|------|---------------------|
| **Trained Hand** |           |      |             |      |                |      |                     |
| Execution time (s) | 1.36      | (0.03) | 1.30        | (0.02) | 1.32           | (0.02) | 2.062               | 0.0869 |
| Error rate (%)    | 13.76     | (2.36) | 5.08        | (1.18) | 5.61           | (1.70) | 7.286               | 0.0022 |
| Force (N)         | 5.99      | (0.34) | 5.65        | (0.29) | 6.85           | (0.22) | 4.627               | 0.0157 |
| **Untrained Hand** |           |      |             |      |                |      |                     |
| Execution time (s) | 1.40      | (0.04) | 1.32        | (0.02) | 1.31           | (0.01) | 4.506               | 0.0174 |
| Error rate (%)    | 15.43     | (2.03) | 6.50        | (1.23) | 5.75           | (1.30) | 11.864              | 0.0001 |
| Force (N)         | 5.69      | (0.26) | 5.50        | (0.27) | 6.71           | (0.29) | 5.718               | 0.0066 |
Table 5: Mirroring of digit forces during fMRI.

Table shows mean (± standard error) of the average force range (maximum – minimum) and standard deviation of the passive (non-moving) hand. Results were split depending on whether the trained hand or the untrained hand was the passive hand. The correlation between the forces was calculated between the force time series of the matching digits of the active and passive hands. The last column indicates the F-test comparing the 3 groups.

|                      | Sham mean | SE   | Bi-tDCS mean | SE   | RP-Bi-tDCS mean | SE   | ANOVA across groups |
|----------------------|-----------|------|---------------|------|-----------------|------|---------------------|
| **Trained Hand**     |           |      |               |      |                 |      |                     |
| Force range [N]       | 0.13      | (0.01)| 0.14          | (0.01)| 0.15            | (0.03)| 0.356               | 0.703 |
| Standard deviation [N]| 0.03      | (0.00)| 0.03          | (0.00)| 0.04            | (0.01)| 0.497               | 0.612 |
| Correlation of force traces | 0.07     | (0.002)| 0.07         | (0.05)| 0.03            | (0.03)| 0.387               | 0.682 |
| **Untrained Hand**    |           |      |               |      |                 |      |                     |
| Force range [N]       | 0.12      | (0.01)| 0.12          | (0.01)| 0.13            | (0.02)| 0.293               | 0.745 |
| Standard deviation [N]| 0.03      | (0.00)| 0.03          | (0.00)| 0.03            | (0.01)| 0.260               | 0.773 |
| Correlation of force traces | 0.09     | (0.02)| 0.05          | (0.03)| 0.02            | (0.03)| 1.981               | 0.154 |