tDCS modulates effective connectivity during motor command following; a potential therapeutic target for disorders of consciousness

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Abstract

Transcranial direct current stimulation (tDCS) is attracting increasing interest as a potential therapeutic route for unresponsive patients with prolonged disorders of consciousness (PDOC). However, research to date has had mixed results. Here, we propose a new direction by directly addressing the mechanisms underlying lack of responsiveness in PDOC, and using these to define our targets and the success of our intervention in the healthy brain first. We used fMRI to characterise the effects of tDCS on brain activity and dynamics during command following, a task typically used to clinically assess awareness. Anodal tDCS over M1 and cathodal tDCS over the cerebellum led to long-range changes in thalamo-cortical coupling associated with the ability to produce motor responses to command. This suggests that tDCS may allow PDOC patients to overcome the motor deficits at the root of their reduced responsiveness, improving their rehabilitation options and quality of life as a result.

Introduction

Transcranial direct current stimulation (tDCS) is a non-invasive brain stimulation technique that is gaining popularity as a therapeutic option for complex clinical conditions for which no other alternatives are available¹. Among these, a paradigmatic case is that of prolonged disorders of consciousness (PDOC), such as the vegetative (VS) and the minimally conscious state (MCS). PDOC are characterised by catastrophic disabilities that are in many cases permanent², and the small number of therapies available have demonstrated very limited success at improving outcome³. In response to this, over the last 5 years the field has seen a sharp rise in tDCS trials on PDOC⁴. These have typically targeted the left dorso-lateral prefrontal cortex (DLPFC), in an attempt to restore some residual level of awareness, but have only had mixed success. While several studies reported the emergence of new behaviours indicative of awareness in subsets of PDOC patients following tDCS (see e.g.⁵), many others have failed to elicit any clinical changes or indeed led to undesired changes⁶. Individual responses to tDCS are well known for their heterogeneity even in healthy populations⁷, and we can expect an even higher variability in PDOC, where the specific aetiology and mechanisms of damage result in marked differences in brain atrophy and tissue microstructure across patients. However, in this particular case, we argue that these difficulties are further exacerbated by our limited understanding of how conscious awareness is supported in the brain, which preclude the identification of effective targets for stimulation. Indeed, while we know that consciousness requires sustained rich neural dynamics in fronto-parietal and thalamo-cortical networks^{8,9}, the specific pattern of activity that would need to be restored in PDOC patients and how this can inform the selection of stimulation targets remains an elusive question.

Here we propose a different approach, wherein we switch the focus from the consciousness disorder itself to the patients' ability to produce voluntary behavioural responses¹⁰. In doing so, we target a cognitive process that is much better understood, not only in terms of its neurophysiology but also which specific tDCS modulations can maximise behavioural changes⁷. In addition, recent voices have emphasised the importance of addressing the fundamentals of any tDCS intervention in well-controlled studies in healthy individuals before a clinical application with meaningful effects can be produced and clinically tested⁷. In line with this, we thus focus on characterizing tDCS responses in the healthy brain, while keeping our methods translatable to PDOC patients. Clinical assessments of PDOC use the patient's ability to follow commands as a proxy measure for their awareness. Crucially, it is well known that a significant number of PDOC patients retain a much greater deal of awareness than can be expected from their clinical diagnosis and are simply unable to demonstrate this with overt purposeful (motor) responses in response to commands^{10,11}. We have recently shown that this lack of behavioural responsiveness is associated with specific impairments within the motor system that result in reduced excitatory coupling between the thalamus and the primary motor cortex (M1)^{12,13}. On this basis we hypothesise that interventions to enhance the flow of information between the thalamus and motor

cortices will provide patients with a renewed level of control over their external behaviour and increase their behavioural responsiveness as a result.

In this study, we use dynamic causal modelling (DCM) of fMRI data to explore whether tDCS can indeed modulate motor thalamo-cortical coupling during simple voluntary responses to command in the healthy brain. We report two separate experiments targeting M1 and the cerebellum respectively. While there is strong evidence that tDCS applied to M1 (henceforth referred to as M1-tDCS) leads to local polarity-specific changes in M1 excitability¹⁴ and BOLD signal¹⁵, little is known about whether it can also influence coupling between other nodes of the motor network. Similarly, there is evidence that cerebellar tDCS (cb-tDCS) is able to modulate cerebellar brain inhibition (CBI)¹⁶, the natural inhibitory tone the cerebellum exerts over M1. Given that the cerebellum is structurally connected to M1 via a thalamic relay, it would follow that the previously reported effects of cb-tDCS on CBI should be mediated by the thalamus. However, no studies have directly investigated how cb-tDCS affects the coupling in this cerebellar-thalamo-M1 axis. Furthermore, no study to date has assessed the effects of either M1- or cb-tDCS on the activity and dynamics of the motor network *during* simple motor command-following. We hypothesised that: (a) anodal M1-tDCS will increase excitation in the motor network and lead to an increased excitatory output from thalamus to M1 during command-following; and (b) cathodal cb-tDCS will reduce inhibition in the thalamus and also result in increased excitation from thalamus to M1. We show that anodal M1-tDCS increases thalamic activity and excitation while cathodal cb-tDCS increases excitation from thalamus to M1. Combined, our two experiments demonstrate that tDCS can successfully modulate long range thalamo-cortical dynamics during motor command following via targeting of cortical regions. Previous research has identified a relative structural preservation of M1-striatal-thalamic and dentate-thalamic pathways in PDOC patients¹³. This suggests that both pathways may be viable routes to target the thalamus in this group.

Results

Experiment 1 - Effects of M1-tDCS on brain activation and dynamics

The aim of this experiment was to assess whether M1-tDCS can modulate thalamo-cortical coupling during a simple command following task. Participants received anodal, cathodal, and sham stimulation sessions in a counterbalanced order while in the MRI scanner. In each session, they completed a simple command-following task before and after 20 minutes of tDCS stimulation at 1mA. Briefly, they performed discrete thumb abduction-adduction movements with their right hand in response to auditory cues (see full description in Fig. 1 and Methods section). The use of a simple task enables both the direct translation of this paradigm to PDOC patients as well as the study of tDCS-induced activation changes independent of performance. Similarly, we delivered all stimulation at rest, without the participant engaging in any motor (or other type of) task, as performing a task during stimulation would not be feasible in PDOC patients themselves.

First, we explored the effects of tDCS on brain activation during command-following. For this, we performed a factorial analysis on the individual activation maps for our thumb-movement task (see also the canonical task activation in Fig. 3). This revealed a significant interaction between polarity (anodal, cathodal, and sham) and time (pre-, post-tDCS) on the left M1, supplementary motor area (SMA), and the left thalamus (FWE-corrected $p < 0.0125$; see Table 1 and Fig. 4). Subsequent pairwise interactions revealed that anodal stimulation increased activity in M1, SMA and thalamus as compared to both cathodal stimulation and sham. In turn, cathodal stimulation compared to sham led to a decrease in brain activity in M1 and SMA, but also an increase in thalamic activation (FWE-corrected $p < 0.0125$). Note that this last comparison also revealed increased activation on M1, but this was restricted to 2 discrete voxels and thus we consider its functional significance as negligible.

We then used dynamic causal modelling (DCM), to characterise the effects of tDCS on the neural dynamics across a network including the 3 regions of interest identified above (left M1, SMA, left thalamus) as well as the right cerebellum. DCM is a framework for Bayesian modelling of brain dynamics, which allows the inference of hidden (unobserved) neuronal states from measured brain activity¹⁷. First, we performed Bayesian Model Reduction (BMR) over nested parameters, with a 95% posterior probability threshold (free-energy), to identify the set of driving inputs that maximised model evidence across all sessions (irrespective of time and polarity). This provided very strong evidence in favour of including driving inputs to M1 and SMA only ($P_p > 99\%$), and we therefore used these to create DCMs for subsequent analyses. We then defined a fully connected 4-node model in which all self- and between-region connections were switched on for each session. The effect of thumb movements entered the model as modulatory input on the self-connection of each region, as this is recommended to improve both parameter identifiability and biological interpretability¹⁷. After creating this starting model, we used Bayesian Inference to invert the individual model for each subject and estimate the parameters that maximise the explanation of data while minimising complexity. Finally, we applied Parametric Empirical

Bayes (PEB) to assess the effects of M1-tDCS on these parameters. Briefly, PEB is a hierarchical Bayesian framework for group-level modelling of effective connectivity, that allows evaluation of both group effects and between-subject variability over DCM parameters (see¹⁸ for a full description). Finally, we used Bayesian Model Reduction (BMR) to search over the reduced models, followed by Bayesian Model Average (BMA) to calculate the average connectivity parameters¹⁸.

We found that anodal stimulation of M1 reduced self-inhibition in the thalamus and led to a more inhibitory output from cerebellum to M1, compared to both sham and cathodal stimulation. Additionally, as compared to sham, anodal stimulation increased inhibition in all outputs from M1 to the rest of the network but reduced inhibition from cerebellum to thalamus, as well as in SMA and cerebellar self-connections. These changes were however not polarity specific. In turn, cathodal stimulation increased excitation from thalamus to SMA, both as compared to sham and to anodal stimulation. Additionally, as compared to sham, cathodal stimulation led to an increase in inhibition from both M1 and cerebellum to SMA, an increase in excitation from thalamus to M1, and a reduction in self-inhibition in SMA. In terms of task modulations, cathodal M1 stimulation increased the modulatory input from the task on M1 (i.e., increased M1 self-inhibition) both as compared to anodal stimulation and sham, and decreased the modulatory input from the task on SMA as compared to anodal stimulation (see Fig. 5).

Experiment 2 - Effects of cb-tDCS on brain activation and causal dynamics

CBI plays an important role in initiating and controlling voluntary movement¹⁹. To our knowledge there are no previous studies of the effects of cb-tDCS on connectivity and coupling across the motor network during a simple motor task. The aim of this experiment was to assess whether cb-tDCS can modulate thalamo-cortical coupling during a simple command following task. The design and analyses in this experiment mirrored those for Experiment 1, with the exception of using a tDCS montage to target the cerebellum, with an intensity of 1.85 mA (see methods)²⁰.

In terms of brain activity during command following, our factorial analysis revealed a significant interaction between polarity and time (pre- vs post-tDCS) in the cerebellum, thalamus, and M1, as well as a small area (3 voxels) in SMA (FWE-corrected $p < 0.0125$; see Table 2 and Fig. 4). Subsequent pairwise interactions revealed that cathodal stimulation led to an increase in brain activity in both the cerebellum and thalamus when compared to sham or anodal stimulation. Additionally, cathodal cb-tDCS decreased M1 and SMA activation as compared to sham. However, the decrease in SMA activity was limited to 2 voxels and thus is likely to have negligible functional significance. In turn, anodal stimulation led to small areas of increased activation in the cerebellum (3 voxels) and M1 (4 voxels).

In terms of effective connectivity, as predicted, cathodal stimulation led to increased excitation from thalamus to M1 both as compared to sham and anodal stimulation. In addition, it increased M1 self-inhibition as compared to sham but to a lesser extent than anodal stimulation. Finally, it increased inhibition from M1 to SMA both as compared to sham and anodal stimulation (Fig. 5). When compared directly with anodal stimulation, cathodal cb-tDCS also decreased cerebellar self-inhibition and increased excitation from thalamus to SMA. Additionally, cathodal stimulation increased inhibition from M1 to cerebellum and from cerebellum to thalamus, and increased excitation from SMA to both thalamus and cerebellum, and from cerebellum to M1, as compared to sham. However, none of these changes were significant when compared with anodal stimulation. Finally, cathodal cb-tDCS decreased the effect of the task on SMA both as compared to sham and anodal stimulation. In contrast, anodal stimulation, when compared to sham and cathodal stimulation, led to increased self-inhibition in M1 and thalamus, reduced self-inhibition in SMA, as well as increased excitation from M1 to SMA. Additionally, anodal stimulation increased excitation from SMA to thalamus and cerebellum, and increased inhibition from M1 to cerebellum when compared to sham, but these changes were not polarity specific (i.e., did not reach statistical significance in the comparison between anodal and cathodal stimulation).

Experiments 1 and 2 - Effects of tDCS on behaviour

To characterise any potential effects of tDCS on motor behaviour we calculated the reaction time, mean velocity and peak acceleration for each discrete movement performed in response to the auditory cues and averaged the values across the full run for each session. We then conducted an ANOVA with polarity and time (pre- vs post-tDCS) as factors for each of the measures. As expected, we did not find any interactions (polarity x time) for any of the metrics considered in Experiment 1 nor 2. In Experiment 2 only, we found a small main effect of time, with reaction times appearing 2 ms faster in the second run as compared to baseline (pre-: $0.30\text{ms} \pm 0.04$ vs post-tDCS: $0.28\text{ms} \pm 0.04$; $p < 0.001$, $\eta_p^2 = 0.5$). See Supplementary Table 1.

Experiments 1 and 2 - Blinding

In order to assess whether our blinding protocol was successful, we used McNemar's test to assess whether the number of correct judgements across the group about whether they had received tDCS or not was different between real stimulation and sham stimulation sessions. We found no significant differences in the number of times that sham and active stimulation sessions were perceived as real in either experiment, suggesting that participants' experiences did not differ between active and sham stimulation sessions and blinding was successful (see Supplementary Table 2).

Discussion

Efforts to use tDCS as a therapeutic intervention in PDOC have had mixed success to date. While some studies showed very promising clinical improvements, many others failed to show any effects even after repeated sessions²¹. The field is thus unable to reach a consensus about whether tDCS would or would not be a feasible therapeutic avenue for this patient group as a result. Most research to date has focused on targeting the left frontal cortex, in an attempt to engage non-specific networks involved in arousal and awareness. Here, we propose a new therapeutic direction that directly addresses the neural mechanisms that support measurable changes in behavioural responses after tDCS at the level of functional thalamo-cortical coupling within the motor network¹².

Our results provide the first evidence that tDCS over motor areas can distally modulate brain activity and causal dynamics in thalamo-cortico-cerebellar loops (beyond the immediate target area of stimulation) *during* behavioural command following, even when the stimulation is delivered at rest. In Experiment 1, anodal stimulation over M1 increased, in a polarity specific manner, not only the local task-induced activation in M1, but also the thalamus. Our DCM analyses revealed that these changes are likely explained by reduced thalamic self-inhibition. In Experiment 2, cathodal cerebellar stimulation also increased task-induced brain activity locally (i.e., in the cerebellum) as well as distally in the thalamus. Crucially, these changes translated into an increased excitatory influence from thalamus to M1. Taken together, these experiments demonstrate that it is possible to influence thalamo-cortical coupling indirectly via targeting *surface* (easily accessible) regions in the motor network. More importantly, they suggest that this could be a viable route to elicit clinically relevant changes in PDOC. Indeed, we designed our command-following task to emulate the approach that is routinely used in clinical settings to assess awareness after severe brain injury; namely asking the patient to perform a discrete movement in response to a verbal command²². This resulted in a task that was insensitive to potential tDCS modulations of behaviour in healthy participants but allowed us to study the neural effects of tDCS independently of performance, permitting us to draw more direct comparisons to the PDOC population. Further, we delivered stimulation at rest to increase the translatability of our results to unresponsive PDOC patients. It is important to highlight that the aim here was not to improve motor control in the healthy brain. Instead, we built upon convincing evidence that the thalamus is greatly inhibited in PDOC due to both structural and functional damage^{23–25}, resulting in less cortical excitation²⁵. Our focus thus lay on compensating for this thalamic over-inhibition instead of enhancing normal function. We have previously shown that increased thalamic activity and excitation, as well as increased excitatory thalamus-M1 coupling facilitates the production of motor responses to command in tasks like the one we used here¹². We now show that anodal tDCS over M1 and cathodal tDCS over the cerebellum can each successfully modulate these dynamics and we propose that they may allow PDOC patients to overcome

motor control deficits at the root of their diminished behavioural responsiveness^{12,13}. This in turn would allow more patients to demonstrate their true level of awareness, especially in those affected by so called cognitive motor dissociations¹⁰. Alongside ensuring that each patient receives an appropriate diagnosis, this increased responsiveness can also have important implications for prognosis by facilitating patients' engagement with rehabilitation²⁶. Moreover, regaining some level of control over their thumb would facilitate the use of assistive devices (including those for communication), which could have an enormous impact on their quality of life. Indeed, to further increase the clinical relevance of our study, we focused on thumb movements, as they are affected by spasticity in fewer PDOC patients and with less severity as compared to other fingers²⁷.

Importantly, our results suggest two potential routes to target the thalamo-M1 axis, providing some flexibility to adapt the tDCS montage to the specific pattern of injuries present in each individual patient. Crucially, while many PDOC patients present localised structural damage to the white matter fibres connecting thalamus and M1^{12,13}, this damage is partial instead of a complete deafferentation¹³. This suggests the remaining pathways may be amenable to therapeutic intervention. In contrast, the white matter pathways connecting the cerebellum with the thalamus appear relatively preserved¹³, suggesting that this may be a feasible route into the thalamus in the majority of PDOC patients. We have previously argued that the relative preservation of this pathway, in the context of damage to the thalamus and the white matter fibres connecting thalamus to M1, may be contributing to excessive thalamic inhibition¹³. As discussed above, our current results show that cathodal cb-tDCS may be able to successfully counteract this. It is important to acknowledge here that, while both anodal M1 and cathodal cb-tDCS successfully modulated thalamic activity, there were differences in their respective effects over M1 activity and the thalamo-M1 dynamics. We include below discussion of potential compensatory mechanisms that may explain these effects. We also note the possibility of simultaneous anodal-M1 and cathodal-cerebellar stimulation, although we have not tested this montage. In any case, further studies in PDOC patients themselves are required to test which of these modulations has greater therapeutic effect and for which specific patients. More broadly, while our results provide a robust proof-of-principle for the use of motor tDCS in PDOC, the specific dose, duration, and number of sessions required to induce reliable neural and behavioural changes in PDOC patients needs to be established. To our knowledge, only 3 studies have targeted motor areas with tDCS in PDOC^{28–30}, in sharp contrast with the many others that have focused on the DLPFC, and currently represents the main direction in the field. These 3 motor studies included a combined total of 40 patients (14 VS and 26 MCS). Their small sample sizes, key differences in specific montages and stimulation parameters, alongside the focus on behaviour instead of neural markers, preclude us from drawing direct comparisons with our study. In addition, while we are satisfied that we were able to identify the optimal location of the electrodes on the scalp to target the desired regions in our study, this is a much more challenging task in patients with severe brain injury, where large macrostructural changes will affect the relative position of the brain structures of interest in respect to the scalp. Nevertheless, PDOC studies provided preliminary evidence that M1 and cerebellar tDCS are well

tolerated in this patient group and can indeed lead to specific improvements in motor responsiveness in a subset of patients (as indexed by increases in the motor and auditory CRS-R subscales).

Beyond the immediate implications for the rehabilitation of PDOC patients, our results speak for the ability of tDCS to influence long-range dynamics in the motor network during movement execution. The field of non-invasive brain stimulation has recently been tainted by a certain level of scepticism towards the effectiveness of tDCS, with some questioning whether it is indeed capable of modulating brain function at all³¹. The increasing number of well controlled imaging and electrophysiological studies has provided reassurance that tDCS can indeed modulate cortical regions under the electrodes. In the specific case of M1 stimulation, this is now well established. Here, we take this argument one step further, demonstrating that it can also lead to widespread distal modulations of cortico-subcortical loops when participants are engaged in a relevant cognitive task, and that such modulations do not require the participant to engage with the said task while receiving the stimulation itself. Specifically, our predicted changes to thalamo-cortical activity and dynamics induced by anodal M1-tDCS (as discussed above), are consistent with, and expand, the now widely reported effects on M1 excitability¹⁴, and well as more recently described changes to BOLD signal^{15,32,33} and functional connectivity at rest^{34–36}. In contrast, the effect of cerebellar tDCS on neural activity and dynamics is much less understood. As discussed above, cathodal cb-tDCS increased thalamic activation and its afferent excitation over M1. In contrast, anodal stimulation led to increased self-inhibition in both M1 and thalamus. These findings demonstrate that tDCS is able to modulate cerebellar-brain inhibition (CBI) in a polarity specific manner, in agreement with previous electrophysiological reports¹⁶, as well as a recent report of local increased activation in the dentate nuclei after cathodal cb-tDCS during simple finger tapping³⁷. Furthermore, for the first time, we provide a window into the specific functional dynamics mediating these effects.

Interestingly, against our prediction, cathodal tDCS over M1 also led to an increase in thalamic activation (albeit to a lesser extent than anodal stimulation), and in excitation from thalamus to M1, as compared to sham. These changes further support the already described complex effects that characterise this polarity.⁷ Importantly, these changes were accompanied by the expected reduced activation in cortical regions and no reductions in motor performance in the task. We believe this suggests that the thalamic changes reflect a compensatory mechanism to overcome cortical inhibition caused by cathodal M1-tDCS and maintain an acceptable level of motor performance. This is in line with earlier animal models suggesting sustained effects of tDCS that are characterised by the system trying to compensate and normalise its activity to baseline levels (see³⁸ as discussed in³⁹). Interestingly, cathodal M1-tDCS also increased the modulatory effect of the task over M1, which further supports this compensatory hypothesis. Similarly, in Experiment 2, cathodal stimulation over the cerebellum led to the expected increases in thalamic activation and excitatory output to M1, but also an unexpected reduction in M1 activation that was explained by increased self-inhibition. Once again, tDCS did not alter behavioural performance and thus we believe this cortical reduction also compensated for the excess excitation

coming from the thalamus. Alongside determining whether these changes have a therapeutic effect, neuroimaging studies of tDCS in PDOC will help elucidate whether these effects are indeed compensatory or can alter behaviour when a motor deficit is present.

In summary, our results indicate that tDCS can successfully modulate thalamo-cortical dynamics underlying behavioural responsiveness during command following. It is yet to be tested whether these effects can be replicated in PDOC patients themselves and whether this will result in measurable clinical effects. However, our methodology can be directly applied to investigate this, and in doing so, it opens new avenues to explore the mechanisms of tDCS interventions in this challenging population.

Methods

Participants

Forty-nine right-handed healthy volunteers participated in the study (15 men, 34 women; mean age 25 ± 4 years). We recruited all participants from the University of Birmingham, using the local Research Participation Scheme and advertisements across campus. We pre-screened all participants before recruitment to confirm their eligibility to safely take part in MRI and tDCS experiments. All reported no previous history of neurological and/or psychiatric disorders, no personal or family history of epilepsy, no use of psychoactive drugs, and had normal or corrected vision. Additionally, we instructed them to be well hydrated and well slept, with no alcohol or coffee consumed during the 24 hours prior to the testing session, to be in keeping with brain stimulation safety regulations.⁴⁰ The University of Birmingham's Science, Technology, Engineering and Mathematics Ethical Review Committee approved the study and all participants gave written informed consent prior participation. We compensated participants with £110 or the equivalent in course credits.

Experiment 1 included 26 participants (8 male, 18 female; mean age 23 ± 4 years), from whom 22 completed all 3 sessions. We further discarded data from one participant due to failure to comply with the task instructions, resulting in a final sample of 21 to be included in the analysis (8 male, 13 female; mean age mean: 23 ± 4 years). *Experiment 2* included 23 participants (7 male, 16 female; mean age: 27 ± 4 years), from whom 22 completed all 3 sessions. We excluded one further participant due to an acquisition error in one of the sessions that resulted in corrupted files. The final sample consisted of 7 males and 14 females, aged 27 ± 4 years.

General Experimental procedure

All participants completed anodal, cathodal, and sham stimulation sessions, scheduled at least 7 days apart (*Experiment 1*: mean 12 ± 13 ; *Experiment 2*: mean 13 ± 10), and in a counterbalanced order. Both

the participants and the researchers conducting the data analyses were blind to the polarity in each session.

In their first testing session participants provided informed consent for the study and completed the Edinburgh handedness inventory⁴¹. Additionally, before each session, we pre-screened participants to confirm MRI and tDCS safety. After completing these steps, we set up the electrodes in a designated room (see below), and took the participants to the MRI scanner, where we completed the setup of the tDCS system and provided the participants with a joystick to record their responses in the fMRI task (see below). We used the MRI Intercom system to communicate with participants during the experiment. Before and after the stimulation, participants performed an fMRI motor command-following task where they were instructed to execute simple thumb movements (abduction-adduction) in response to auditory cues (see fMRI paradigm below). Finally, to test whether our protocol achieved adequate blinding, participants completed a post-tDCS perceptual scale of their perceived sensations and/or discomfort after each session, and indicated whether they thought they received actual stimulation or sham.

Electrical Stimulation

In both experiments we administered tDCS in the MRI scanner using an MR-compatible NeuroConn DC-Stimulator MR (neuroCare Group GmbH, Germany). We used 5x5 cm² electrodes with electro-conductive paste to improve conduction, and secured them in place using self-adhesive bandage.

Experiment 1. In line with previous studies targeting M1¹⁴, in the anodal sessions we placed the target electrode (anode) centred on the left motor hotspot, as identified by TMS prior to the first MRI session, and oriented approximately at a 45° angle with respect to the midline. We placed the reference electrode (cathode) on the contralateral supraorbital region. We reversed this montage for the cathodal sessions. Half of the sham sessions replicated the anodal montage and the other half the cathodal montage. We used a Magstim BiStim² TMS stimulator paired with Brainsight TMS navigation system (Rogue Research Inc) to identify the motor hotspot in each participant in the first stimulation session, following standard methods⁴².

Experiment 2. We placed the target electrode on the right cerebellar cortex (3 centimetres lateral to theinion, oriented parallel to the midline) and the return electrode on the right buccinator muscle⁴³. The montage was reversed for anodal and cathodal sessions. As above, half of the sham sessions replicated the anodal montage and the other half the cathodal montage.

In both experiments, we used Brainsight to record the coordinates for the target electrode in the first session and used them to locate the electrode position for the subsequent sessions to ensure consistent placement.

During anodal and cathodal sessions, we delivered 20 minutes of stimulation, with 30 seconds of ramp-up and ramp-down periods. During sham, we delivered 30 seconds of stimulation before ramping down to give the sensation of active stimulation, and according to well established protocols to ensure blinding⁴⁴. In *Experiment 1* we stimulated at an intensity of 1mA, as this typically induces tDCS canonical excitatory versus inhibitory effects for anodal and cathodal stimulation respectively^{7,14}. In *Experiment 2*, we stimulated at an intensity of 1.85mA as previously recommended²⁰.

MRI acquisition

We acquired all data on a Philips Achieva 3T system, with a 32-channel head coil, at the Birmingham University Imaging Centre (BUIC).

Experiment 1. fMRI acquisition parameters were as follows: 160 volumes per run, 34 slices, TR = 2000ms, TE = 35ms, matrix size = 80 × 80, voxel size = 3x3x3mm, no gap, and flip angle = 79.1°. Additionally, we acquired a high-resolution, T1-weighted MPRAGE image, for anatomical co-registration, with the following parameters: TR = 7.4ms, TE = 3.5ms, matrix size = 256x256mm, voxel size = 1x1x1mm, and flip angle = 7°. In the same session we collected other anatomical data and resting state fMRI but we did not analyse these within the current study and we will report them in a separate paper.

Experiment 2. fMRI acquisition parameters were as follows: 119 volumes per run, 46 slices, TR = 2700ms, TE = 35ms, matrix size = 80x80, voxel size = 3x3x3, no gap, flip angle = 79.1°. High-resolution, T1-weighted MPRAGE images were also acquired for Experiment 2, with the following parameters: TR = 7.4ms, TE = 3.5ms, matrix size = 256x256, voxel size = 1x1x1, and flip angle = 7°.

fMRI paradigm

We instructed participants to perform a thumb adduction-abduction movement as fast as they could in response to auditory cues (beeps). We presented the beeps in blocks cued by the word 'move' and interspersed with blocks in which the participant was instructed to rest (cued by the word 'relax'). Each 'move' block included 7 beeps presented at a variable interstimulus interval (range 2-3 seconds), in order to avoid prediction effects. The task included 8 blocks of each type, each with a duration of 20 seconds, and for a total duration of 5 minutes and 20 seconds. We instructed the participants to maintain fixation on a white cross displayed in the centre of a black screen throughout the full duration of the task. This, as well as the instructions at the start of the task ("*Start moving your thumb as quickly as you can every time*

you hear a beep. Stay still when you hear "relax". Make sure you keep looking at the fixation cross at all times") were presented via a digital system (Barco F35 AS3D, Norway) that projected the image onto a mirror fixed to the head coil at a visual angle of $\sim 10^\circ$. We delivered all auditory cues with an MR-compatible high-quality digital sound system incorporating noise-attenuated headphones (Avotec Silent Scan®). During 'move' blocks, we recorded thumb movements with an MRI compatible joystick (FORP-932, Current designs INC., PA USA), using 1200 Hz sampling frequency of x and y positions. To facilitate use of the joystick inside the MRI bore, the device was connected to the interface in the control room through an optical cable. For each session, we stabilised the joystick on the participant's torso and stabilised their right thumb using tape. To ensure accurate recordings, we calibrated the joystick before starting the experiment in each session. We used MATLAB 2015b on a Windows 7 computer to deliver all task stimulus and record motion tracking. See Fig. 1.

fMRI preprocessing and GLM analysis

We used SPM12 on MATLAB 2015b (www.fil.ion.ucl.ac.uk/spm) for the preprocessing and analysis of both fMRI datasets. Each dataset was analysed independently but following the same pipeline, as described here. We first followed a standard spatial pre-processing, including realignment, co-registration between the structural and functional data sets, spatial normalization, and smoothing with an 8mm fwhm Gaussian kernel). Additionally, in order to remove potential undesirable effects of physiological noise or participant's motion in the activation maps, we performed single-subject independent component analysis (ICA)⁴⁵ and then applied FMRIB's ICA- based X-noiseifier (FIX)^{46,47} to identify artefactual components and remove them from our fMRI data. We first classified manually all components from a subset of datasets (18 in *Experiment 1* and 23 in *Experiment 2*), ensuring an even coverage of all possible combinations of sessions, times, and polarities. Then, we used these manual labels to train a classifier for each of the studies that we then applied to the remaining datasets in that study. In order to test the accuracy of the automatic component classification, two of the authors (D.F-E for *Experiment 1* and D.A. for *Experiment 2*) independently classified a number of components in the training set (8 datasets for *Experiment 1* and 10 datasets for *Experiment 2*) and cross-checked their manual classification against the automatic classifications performed by FIX. There was a 100% match for 'bad' components between the manual and automatic classification lists.

We performed single-participant fixed-effect analyses using a general linear model in which we modelled each scan to belong to the motor execution (i.e. blocks of thumb movements) or the rest condition. The model also included the realignment factors as effects of non-interest to account for residual motion-related variance. We used high-pass filtering with a cut-off period of 80 seconds to remove slow-signal drifts from the time series. We then set linear contrasts to obtain estimates of the effects of interest for each subject, polarity, and time. Finally, in order to test the effects of tDCS on brain activation, we performed a second level full factorial analysis with polarity (anodal, cathodal, and sham) and time

(before and after tDCS) as factors (total number of sessions = 126 for *Experiment 1* and 126 for *Experiment 2*). When the interaction was significant, we also performed the corresponding pairwise interactions to study the direction of the effects. We report statistically significant voxels as being only those that survive a family-wise error (FWE)-corrected $p < 0.0125$ at the voxel level, on the following regions of interest: left SMA, left precentral gyrus, left thalamus, and right cerebellar lobes IV-V and VIII⁴⁸, using WFU PickAtlas. This includes a Bonferroni correction for the number separate ROIs applied ($0.5/4$)⁴⁹. In addition, we did not include spurious activation, defined as a contrast returning a single significant voxel. We obtained these regions of interest from the Automated Anatomical Labeling atlas⁵⁰. In *Experiment 1*, we had to exclude one participant from the ANOVA due to an acquisition error in one of the sessions that resulted in the most superior slices of the brain being cropped (losing a small section of M1). Note however that this issue did not affect the VOI analyses for the DCM (see section below) and therefore this participant was included in the DCM analyses. See full analysis pipeline in Fig. 2.

DCM analysis

Region selection and timeseries extraction

First, to obtain the canonical pattern of activity on our task for the group in each experiment, we performed second-level one-sample t-tests on the individual contrasts corresponding to the pre-stimulation run acquired in the first chronological session for each participant (Fig. 3). In the resulting map, we identified the group peak of activation for the clusters corresponding to the left M1, SMA, left thalamus, and right cerebellum at an uncorrected $p < 0.001$ (in bold in Table 3). This group-derived coordinates then served as a starting point for searching a nearby local maximum in each individual run. Each of these run-specific local maxima was constrained to be a maximum of 15mm away from the group level peak for the left M1, SMA, and right cerebellum ROIs and a maximum of 9mm away for the left thalamus ROI, and had to exceed a liberal statistical threshold of $p < 0.05$ ¹⁷. The differences in the allowed distance from the group peak accommodated for differences in size of the anatomical boundaries of each region. As recently recommended, when this threshold failed to produce a peak for that region, we iteratively reduced the threshold in 0.05 increments until reaching 0.25. When no peak could be found even at this threshold, we used the original group derived coordinates, as typically done¹⁸. Note that we only used these liberal thresholds for the identification of coordinates to extract our timeseries (feature selection) but not for any statistical analyses. Having identified individual peak coordinates for each run, we extracted timeseries from 4mm radius spherical volumes of interest centred on them.

Individual level DCM specification and definition of model space.

With the extracted timeseries, we specified individual dynamic causal models using the deterministic model for fMRI, one-state per region, bilinear modulatory effects, and mean-centred inputs. We started with a fully connected model in which all self- and between region connections were switched on. The

effect of thumb movements entered the model as modulatory input on the self-connection of each region. Then, we used Bayesian Inference to invert the model for each subject and estimate the parameters that maximise explanation of data while minimising complexity. In addition to the intrinsic connections and modulatory inputs above, DCM requires the specification of driving inputs, which briefly ‘ping’ specific regions in the network at the onset of each block. In order to determine the best set of inputs for our data we first created DCMs that included driving inputs to all 4 regions in our model and applied Parametric Empirical Bayes (PEB) to prune any parameters that were not contributing to the model evidence. Briefly, PEB is a hierarchical Bayesian framework for group-level modelling of effective connectivity, that allows the evaluation of both group effects and between-subject variability over DCM parameters (see¹⁸ for a full description). For this step, we created a second-level PEB modelling the commonalities across all 6 sessions for each participant. These were then fed to a third-level PEB that modelled the commonalities across the group. In addition to the constant encoding the group mean, we included sex, age, and the score in the Edinburgh Handedness Inventory as nuisance regressors (all mean-centered). Finally, we used Bayesian Model Reduction (BMR) to search over the reduced models followed by Bayesian Model Average (BMA) to calculate the average connectivity parameters¹⁸. This step indicated strong support for including driving inputs to cortical regions (M1, and SMA) only (see results for full details) and therefore we re-defined DCMs for all of our participants using these parameters (NB. connections and modulatory inputs were as above).

ANOVA

To test the effects of tDCS on the model parameters (connections and task modulations), we first created 3 second-level PEB models in each participant, which encoded the following pair-wise interactions: (1) greater increases after anodal stimulation as compared to sham (pre-tDCS < post-tDCS x anodal > sham sessions) and (2) greater increases after anodal stimulation as compared to cathodal (pre-tDCS < post-tDCS x anodal > cathodal), and (3) greater increases after cathodal stimulation as compared to sham (pre-tDCS < post-tDCS x cathodal > sham). Note that these contrasts also encode the opposite effects: e.g., PEB 1 can also be interpreted as greater decreases in sham as compared to anodal (pre-tDCS > post-tDCS x anodal < sham). Each subject specific PEB model was then entered into one of 3 third-level PEBs that encoded the commonalities across the group (mean) for each pairwise interaction, as well as sex, age, and handedness score.

We then used BMR and BMA to prune connections that do not contribute to the model evidence and estimate the parameters across all models for each of the connections that remain switched on. We thresholded our BMA results at a posterior probability > 95% (which is equivalent to a Bayes factor of 3).¹⁸

Motion tracking

We performed motion data analysis using a custom script on MATLAB 2017b. First, we calculated the Euclidean distance of the x-y position and applied a low-pass 15Hz filter to the data. We then identified the

beginning and end of the movement by looking at abrupt changes in the signal, using the matlab function *findchangepts*, which, given a vector *x* with *N* elements (in our case containing motion tracking data) returns the index at which the mean of *x* changes most significantly. We used the first and last change detected by *findchangepts* to determine when each movement started and ended. We excluded movements where no changes were detected, which could be due to participants not responding to the task or to the joystick not recording data. In *Experiment 1*, this resulted in the removal of 5 datasets from the motion tracking analysis, due to the joystick malfunctioning during recording in at least one of three sessions. Lastly, we calculated velocity and acceleration at each timepoint between the beginning and end of each movement and obtained the mean velocity and peak acceleration for the trial. Additionally, we calculated reaction time defined as the time occurring between the auditory stimulus (beep) and the onset of the movement. Finally, we averaged these values across each run and computed a 2 (pre- vs post-tDCS) x3 (polarity) repeated measures ANOVA to check for any effect of tDCS on behaviour.

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Author Contributions

DF-E designed the study and obtained funding. DA, RJ, PT, and DFE collected the data. DA and DF-E analysed the data, interpreted the results, prepared the figures, and wrote the manuscript. CRM, PT contributed to the editing of the final draft. All authors approved the content of the manuscript.

References

1. Liu, A. *et al.* Immediate neurophysiological effects of transcranial electrical stimulation. *Nat. Commun.* **9**, (2018).
2. The Multi-Society Task Force on PVS. Medical aspects of the persistent vegetative state (I). *N. Engl. J. Med.* **330**, 1499–1508 (1994).
3. Thibaut, A., Schiff, N., Giacino, J., Laureys, S. & Gosseries, O. Therapeutic interventions in patients with prolonged disorders of consciousness. *Lancet Neurol.* **18**, 600–614 (2019).
4. Bourdillon, P., Hermann, B., Sitt, J. D. & Naccache, L. Electromagnetic Brain Stimulation in Patients With Disorders of Consciousness. *Front. Neurosci.* **13**, 223 (2019).

5. Thibaut, A., Bruno, M.-A., Ledoux, D., Demertzi, A. & Laureys, S. tDCS in patients with disorders of consciousness: Sham-controlled randomized double-blind study. *Neurology* (2014).
6. Martens, G. *et al.* Behavioral and electrophysiological effects of network-based frontoparietal tDCS in patients with severe brain injury: A randomized controlled trial. *NeuroImage Clin.* **28**, (2020).
7. Parkin, B. L., Ekhtiari, H. & Walsh, V. F. Non-invasive Human Brain Stimulation in Cognitive Neuroscience: A Primer. *Neuron* **87**, 932–945 (2015).
8. Luppi, A. I. *et al.* Consciousness-specific dynamic interactions of brain integration and functional diversity. *Nat. Commun.* **10**, 4616 (2019).
9. Demertzi, A. *et al.* Human consciousness is supported by dynamic complex patterns of brain signal coordination. *Sci. Adv.* **5**, eaat7603 (2019).
10. Schiff, N. D. Cognitive Motor Dissociation Following Severe Brain Injuries. *JAMA Neurol.* **72**, 1413–1415 (2015).
11. Fernández-Espejo, D. & Owen, A. M. Detecting awareness after severe brain injury. *Nat. Rev. Neurosci.* **14**, 801–809 (2013).
12. Fernandez-Espejo, D., Rossit, S. & Owen, A. M. A Thalamocortical Mechanism for the Absence of Overt Motor Behavior in Covertly Aware Patients. *JAMA Neurol.* **72**, 1–9 (2015).
13. Stafford, C. A., Owen, A. M. & Fernández-Espejo, D. The neural basis of external responsiveness in prolonged disorders of consciousness. *NeuroImage Clin.* **22**, 101791 (2019).
14. Nitsche, M. A. & Paulus, W. Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. *J. Physiol.* **527**, 633–639 (2000).
15. Stagg, C. J. *et al.* Modulation of movement-associated cortical activation by transcranial direct current stimulation. *Eur. J. Neurosci.* **30**, 1412–1423 (2009).

16. Galea, J. M., Jayaram, G., Ajagbe, L. & Celnik, P. Modulation of cerebellar excitability by polarity-specific noninvasive direct current stimulation. *J. Neurosci. Off. J. Soc. Neurosci.* **29**, 9115–9122 (2009).
17. Zeidman, P. *et al.* A guide to group effective connectivity analysis, part 1: First level analysis with DCM for fMRI. *NeuroImage* **200**, 174–190 (2019).
18. Zeidman, P. *et al.* A guide to group effective connectivity analysis, part 2: Second level analysis with PEB. *NeuroImage* **200**, 12–25 (2019).
19. Stefanescu, M. R. *et al.* A 7T fMRI study of cerebellar activation in sequential finger movement tasks. *Exp. Brain Res.* **228**, 243–254 (2013).
20. Jalali, R., Chowdhury, A., Wilson, M., Miall, R. C. & Galea, J. M. Neural changes associated with cerebellar tDCS studied using MR spectroscopy. *Exp. Brain Res.* **236**, 997–1006 (2018).
21. Aloï, D., Incisa della Rocchetta, A., Ditchfield, A., Coulborn, S. & Fernandez-Espejo, D. Therapeutic use of transcranial direct current stimulation in the rehabilitation of prolonged disorders of consciousness. *Front. Neurol.*
22. Fernandez-Espejo, D. & Owen, A. M. Detecting awareness after severe brain injury. *Nat. Rev. Neurosci.* **14**, 801–809 (2013).
23. Fernandez-Espejo, D. *et al.* Reductions of thalamic volume and regional shape changes in the vegetative and the minimally conscious states. *J. Neurotrauma* **27**, 1187–1193 (2010).
24. Fernandez-Espejo, D. *et al.* Diffusion weighted imaging distinguishes the vegetative state from the minimally conscious state. *NeuroImage* **54**, 103–112 (2011).
25. Schiff, N. D. Recovery of consciousness after brain injury: a mesocircuit hypothesis. *Trends Neurosci.* **33**, 1–9 (2010).
26. Elliott, L. & Walker, L. Rehabilitation interventions for vegetative and minimally conscious patients. *Neuropsychol. Rehabil.* **15**, 480–493 (2005).

27. Zhang, B. *et al.* Spasticity Management in Persons with Disorders of Consciousness. *PM&R n/a*.
28. Naro, A. *et al.* Cortical connectivity modulation induced by cerebellar oscillatory transcranial direct current stimulation in patients with chronic disorders of consciousness: A marker of covert cognition? *Clin. Neurophysiol.* **127**, 1845–1854 (2016).
29. Martens, G. *et al.* Single tDCS session of motor cortex in patients with disorders of consciousness: a pilot study. *Brain Inj.* **33**, 1679–1683 (2019).
30. Straudi, S. *et al.* Bilateral M1 anodal transcranial direct current stimulation in post traumatic chronic minimally conscious state: a pilot EEG-tDCS study. *Brain Inj.* **33**, 490–495 (2019).
31. Filmer, H. L., Mattingley, J. B. & Dux, P. E. Modulating brain activity and behaviour with tDCS: rumours of its death have been greatly exaggerated. *Cortex* (2019) doi:10.1016/j.cortex.2019.10.006.
32. Baudewig, J., Nitsche, M. A., Paulus, W. & Frahm, J. Regional modulation of BOLD MRI responses to human sensorimotor activation by transcranial direct current stimulation. *Magn. Reson. Med. Off. J. Soc. Magn. Reson. Med. Soc. Magn. Reson. Med.* **45**, 196–201 (2001).
33. Jang, S. H. *et al.* The effect of transcranial direct current stimulation on the cortical activation by motor task in the human brain: an fMRI study. *Neurosci. Lett.* **460**, 117–120 (2009).
34. Polania, R., Paulus, W. & Nitsche, M. A. Modulating cortico-striatal and thalamo-cortical functional connectivity with transcranial direct current stimulation. *Hum. Brain Mapp.* **33**, 2499–2508 (2012).
35. Sankarasubramanian, V. *et al.* Transcranial Direct Current Stimulation Targeting Primary Motor Versus Dorsolateral Prefrontal Cortices: Proof-of-Concept Study Investigating Functional Connectivity of Thalamocortical Networks Specific to Sensory-Affective Information Processing. *Brain Connect.* **7**, 182–196 (2017).

36. Cummmiford, C. M. *et al.* Changes in resting state functional connectivity after repetitive transcranial direct current stimulation applied to motor cortex in fibromyalgia patients. *Arthritis Res. Ther.* **18**, (2016).
37. Küper, M. *et al.* Cerebellar transcranial direct current stimulation modulates the fMRI signal in the cerebellar nuclei in a simple motor task. *Brain Stimulat.* **12**, 1169–1176 (2019).
38. Reato, D., Rahman, A., Bikson, M. & Parra, L. C. Low-Intensity Electrical Stimulation Affects Network Dynamics by Modulating Population Rate and Spike Timing. *J. Neurosci.* **30**, 15067–15079 (2010).
39. Jackson, M. P. *et al.* Animal Models of transcranial Direct Current Stimulation: Methods and Mechanisms. *Clin. Neurophysiol. Off. J. Int. Fed. Clin. Neurophysiol.* **127**, 3425–3454 (2016).
40. Antal, A. *et al.* Low intensity transcranial electric stimulation: Safety, ethical, legal regulatory and application guidelines. *Clin. Neurophysiol. Off. J. Int. Fed. Clin. Neurophysiol.* **128**, 1774–1809 (2017).
41. Oldfield, R. C. The assessment and analysis of handedness: The Edinburgh inventory. *Neuropsychologia* **9**, 97–113 (1971).
42. Rossini, P. M. *et al.* Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: Basic principles and procedures for routine clinical and research application. An updated report from an I.F.C.N. Committee. *Clin. Neurophysiol. Off. J. Int. Fed. Clin. Neurophysiol.* **126**, 1071–1107 (2015).
43. Galea, J. M., Vazquez, A., Pasricha, N., de Xivry, J.-J. O. & Celnik, P. Dissociating the roles of the cerebellum and motor cortex during adaptive learning: the motor cortex retains what the cerebellum learns. *Cereb. Cortex N. Y. N 1991* **21**, 1761–1770 (2011).
44. Woods, A. *et al.* A technical guide to tDCS, and related non-invasive brain stimulation tools. *Clin. Neurophysiol. Off. J. Int. Fed. Clin. Neurophysiol.* **127**, 1031–1048 (2016).

45. Beckmann, C. F. & Smith, S. M. Probabilistic independent component analysis for functional magnetic resonance imaging. *IEEE Trans. Med. Imaging* **23**, 137–152 (2004).
46. Griffanti, L. *et al.* ICA-based artefact and accelerated fMRI acquisition for improved Resting State Network imaging. *NeuroImage* **95**, 232–247 (2014).
47. Salimi-Khorshidi, G. *et al.* Automatic Denoising of Functional MRI Data: Combining Independent Component Analysis and Hierarchical Fusion of Classifiers. *NeuroImage* **90**, 449–468 (2014).
48. Stoodley, C. J., Valera, E. M. & Schmahmann, J. D. Functional topography of the cerebellum for motor and cognitive tasks: an fMRI study. *NeuroImage* **59**, 1560–1570 (2012).
49. Roiser, J. P. *et al.* Minimum statistical standards for submissions to Neuroimage: Clinical. *NeuroImage Clin.* **12**, 1045–1047 (2016).
50. Maldjian, J. A., Laurienti, P. J., Kraft, R. A. & Burdette, J. H. An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. *NeuroImage* **19**, 1233–1239 (2003).

Fig 1. Experimental design. **(A)** Participants performed a simple behavioural command following task in the MRI scanner (CF-fMRI) before and after receiving 20 minutes of tDCS, whereby they move their right thumb in response to auditory cues (beeps). The task alternated 8 blocks of movement interspersed with rest blocks (all blocks were 20 seconds long for a total of 5 minutes 20 seconds). The beginning of each block was cued by the auditory words 'move' (movement blocks) or 'relax' (rest blocks). In each 'move' block the participants were instructed to perform 7 discrete thumb adduction-abduction movements as fast as they could in response to beeps that appeared at intervals ranging from 2-3 seconds, and while keeping their gaze fixated on a fixation cross displayed in the centre of a black screen. Their movements were recorded with an MRI compatible joystick, using 1200 Hz sampling frequency of x and y positions **(B)**. All participants received anodal, cathodal, and sham stimulation sessions in a counterbalanced order at least 7 days apart. In Experiment 1, we used a montage that targeted the left primary

motor cortex (M1) with the reference electrode over the contralateral supraorbital region, and delivered our stimulation at 1mA (**C, top inset**). We used TMS to identify the best placement (motor hotspot) of the active electrode in each participant. In Experiment 2, our montage targeted the right cerebellar cortex, with a reference electrode over the right buccinator muscle, and delivered our stimulation at 1.85mA (**C, bottom inset**). (**C**)

D: Computational model showing the electric field distributions in Experiment 1 (left) and Experiment 2 (right), as calculated with SimNIBS3.2.2 on the "Ernie" head model. For the purpose of this simulation, in Experiment 1, we placed the active electrode in the area between Fc1 and C3 to approximate the location of the motor hotspot in our participants, and the passive electrode on Fp2 . In Experiment 2, we placed the active electrode on I2 and the passive electrode over the right buccinator muscle. Note that this model does not consider individual differences in the position of the electrodes or the different tissue compartments across our individual participants and therefore it should be interpreted as an estimate of the canonical field distribution to be expected with our montages.

Fig 2. Analysis pipeline. We followed a standard pre-processing protocol (red panel), followed by fixed-effect general linear model analysis to model the effect of thumb movements in each individual participant (1st-level, green panel). We then conducted a second level full factorial analysis to test the effects of tDCS on brain activation (green panel). In addition, we performed a second-level one-sample t-test on the pre-stimulation run acquired in the first chronological session for each participant to characterise the canonical activation in the task, and define coordinates for the subsequent dynamic causal modelling (DCM) analyses. Finally, we used DCM to assess the effects of tDCS on the causal dynamics within our network of interest (yellow panel). We first built and estimated a fully connected model including left M1, left SMA, left thalamus, and right cerebellum in each participant. Then we applied Parametric Empirical Bayes (PEB) to model each of the three pairwise interactions between polarity and time (i.e., interaction between pre-/post-tDCS and either anodal/cathodal, cathodal/sham, anodal/sham) in each participant (2nd-level, yellow panel). Finally, we created a 3rd-level PEB for each pairwise interaction modelling the average effect across participants. Note that we conducted data analysis for each Experiment individually but following the same protocol, as described above.

Fig 3. Brain activation during command following in the pre-stimulation run corresponding to the first session for each participant. The insets display group general linear model differences between 'move' and 'rest' blocks in Experiment 1 (yellow) and Experiment 2 (light blue). The overlap across experiments appears in green. For display purposes, activation maps are shown at an uncorrected $p < 0.001$ and rendered on a standard template (152 template in MRICroGL). z indicates the Montreal Neurological Institute z coordinate.

Fig 4. Effects of tDCS on brain activation during command following for Experiment 1 (left) and 2 (right). The brain insets display group general linear model interactions between polarity (anodal, cathodal, sham) and time (pre-, post-tDCS) in the individual contrasts modelling brain activation during command following. For display purposes, activation maps are shown at an uncorrected $p < 0.001$ and rendered on a standard template (152 template in MRICroGL). z indicates the Montreal Neurological Institute z coordinate. Bar plots show the estimated effect size and 90% confidence intervals at the peak voxel for each pairwise contrast: greater activation after anodal stimulation as compared to sham (A/S) or cathodal stimulation (A/C), greater activation after cathodal stimulation as compared to sham (C/S) anodal stimulation (C/A). Negative bars instead indicate greater decreases in brain activation for the above mentioned contrasts.

Fig 5. Effects of tDCS on functional neural dynamics with M1 (Experiment 1, top panels) or the cerebellum (Experiment 2, bottom panels) as targets. The left and right panels represent changes after anodal and cathodal stimulation respectively. Red arrows indicate changes in the direction of increased excitation (or reduced inhibition). Blue arrows indicate changes in the direction of increased inhibition (or reduced excitation). Note that self-connections are always inhibitory and thus red indicates a reduction in inhibition rather than an excitatory role per-se. Similarly, modulatory inputs from our command following task on each region increase (blue) or decrease (red) the region's inhibitory tone. Thick lines represent changes that are significant both as compared to the opposite polarity and to sham. Thin lines represent changes that are only significant as compared to the opposite polarity. Dashed lines represent changes that are only significant as compared to sham (not polarity specific). The purple boxes highlight our hypotheses for the M1-thalamus axis: anodal M1-tDCS (top left panel) reduced self-inhibition in the thalamus while cathodal cb-tDCS (bottom right panel) increased excitation from thalamus to M1, both in a polarity specific manner.

Tables

Table 1. Effect of M1-tDCS on brain activation.

	Region	Cluster P	Cluster size	Peak P		F / T	MNI coordinates
Contrast		<i>FWE-corrected</i>	<i>in mm3</i>	<i>FWE-corrected</i>	<i>uncorrected</i>		<i>[x;y;z]</i>
Interaction between polarity and time	M1	<0.001	972	<0.001	<0.001	32.998	[-33;5;41]
	SMA	<0.001	3537	<0.001	<0.001	38.087	[-9;11;53]
				<0.001	<0.001	36.492	[-9;20;56]
				<0.001	<0.001	33.801	[0;14;59]
	Thalamus	<0.001	2997	<0.001	<0.001	49.803	[-3;-16;-1]
				<0.001	<0.001	30.997	[-15;-25;-1]
				<0.001	<0.001	21.596	[-6;-25;-1]
		0.008	162	0.001	<0.001	18.045	[-12;-22;17]
Greater Increase after anodal as compared to cathodal	M1	0.002	432	<0.001	<0.001	6.939	[-33;8;44]
		0.023	54	0.002	<0.001	5.231	[-54;11;32]
	SMA	<0.001	3375	<0.001	<0.001	8.713	[-9;11;53]
				<0.001	<0.001	8.437	[-9;20;56]
				<0.001	<0.001	6.188	[0;20;59]
	Thalamus	<0.001	1107	<0.001	<0.001	6.578	[-15;-22;-1]
		0.009	189	<0.001	<0.001	5.740	[-12;-22;17]
Greater increase after cathodal as compared to sham	M1	0.023	54	0.005	<0.001	4.997	[-30;-25;68]
	Thalamus	0.002	432	<0.001	<0.001	9.683	[-3;-16;-1]
				0.002	<0.001	5.175	[-3;-10;8]
		0.012	135	<0.001	<0.001	5.778	[-18;-28;11]
Greater increase after anodal as compared to sham	M1	0.010	162	0.002	<0.001	5.270	[-36;-7;59]
	SMA	<0.001	2565	<0.001	<0.001	8.093	[0;14;59]
				<0.001	<0.001	5.692	[-6;8;44]
				0.001	<0.001	5.462	[0;5;68]
	Thalamus	<0.001	3294	<0.001	<0.001	7.616	[-15;-25;-1]
				<0.001	<0.001	7.134	[-6;-13;-1]
				<0.001	<0.001	6.457	[-6;-25;-1]

Greater decreases after cathodal as compared to sham	M1	<0.001	1161	<0.001	<0.001	7.469	[-33;5;41]
	SMA	0.003	405	0.001	<0.001	5.354	[-9;23;50]
				0.004	<0.001	5.063	[-9;14;53]

Results from the random effect group analyses on the brain activation during thumb movements to command. We only include results that survive a threshold of $p < 0.0125$ FWE-corrected (NB. This threshold includes Bonferroni correction for the use of 4 ROIs). In addition, we do not include spurious single voxel activations. Abbreviations: FWE, family wise error; MNI, Montreal Neurological Institute; M1, primary motor cortex; SMA, supplementary motor area.

Table 2. Effect of Cb-tDCS on brain activation.

	Region	Cluster P	Cluster size	Peak P		F / T	MNI coordinates
Contrast		<i>FWE-corrected</i>	<i>in mm3</i>	<i>FWE-corrected</i>	<i>uncorrected</i>		<i>[x;y;z]</i>
Interaction between polarity and time	M1	0.004	243	0.000	0.000	22.092	[-30;2;44]
		0.015	81	0.001	0.000	18.028	[-57;2;35]
	SMA	0.015	81	0.000	0.000	18.940	[-9;23;50]
	Thalamus	0.000	810	0.000	0.000	22.509	[-21;-22;-1]
				0.000	0.000	21.877	[-15;-13;11]
	Cerebellum	0.001	486	0.000	0.000	23.885	[9;-55;-22]
				0.000	0.000	22.892	[15;-40;-19]
		0.002	324	0.000	0.000	23.293	[15;-55;-43]
Greater increase after cathodal as compared to sham	Thalamus	0.000	1242	0.000	0.000	6.049	[-15;-13;11]
				0.006	0.000	5.004	[-3;-25;5]
	Cerebellum	0.001	675	0.000	0.000	6.434	[9;-55;-22]
		0.008	189	0.001	0.000	5.436	[18;-55;-43]
Greater increase after cathodal as compared to anodal	Thalamus	0.001	567	0.000	0.000	5.935	[-18;-25;8]
				0.000	0.000	5.870	[-15;-13;11]
		0.023	54	0.003	0.000	5.182	[-21;-25;-1]
	Cerebellum	0.003	351	0.000	0.000	6.771	[15;-55;-43]
		0.004	324	0.000	0.000	6.754	[15;-40;-19]
Greater increase after anodal as compared to sham	M1	0.014	108	0.002	0.000	5.272	[-57;2;35]
	Cerebellum	0.018	81	0.002	0.000	5.221	[9;-55;-22]
Greater decrease after cathodal as compared to sham	M1	0.005	270	0.000	0.000	6.142	[-30;2;44]
	SMA	0.023	54	0.010	0.000	4.908	[-9;23;50]

Results from the random effect group analyses on the brain activation during thumb movements to command. We only include results that survive a threshold of $p < 0.0125$ FWE-corrected (NB. This threshold includes Bonferroni correction for the use of 4 ROIs). In addition we do not include spurious single voxel activations. Abbreviations: FWE, family wise error; MNI, Montreal Neurological Institute; M1, primary motor cortex; SMA, supplementary motor area.

Table 3. Canonical activation during command-following

	Region	Cluster P	Cluster size	Peak P	T	MNI coordinates
		<i>FWE-corrected</i>	<i>in mm3</i>	<i>uncorrected</i>		<i>[x;y;z]</i>
Experiment 1	<i>M1</i>	<0.001	6264	<0.001	6.770	[-33;-13;62]
				<0.001	6.767	[-33;-19;56]
		0.966	81	<0.001	4.751	[-15;-4;68]
		0.903	162	<0.001	4.704	[-57;5;29]
		0.927	135	<0.001	4.324	[-54;5;14]
	<i>SMA</i>	<0.001	6426	<0.001	8.703	[0;-7;65]
	<i>Thalamus</i>	0.260	864	<0.001	6.330	[-12;-22;5]
	<i>Cerebellum</i>	0.022	2187	<0.001	7.137	[15;-55;-22]
Experiment 2	<i>M1</i>	0.326	702	<0.001	5.066	[-24;-19;74]
		0.983	81	<0.001	4.565	[-54;5;14]
		0.983	81	<0.001	4.251	[-15;-4;68]
		0.195	918	<0.001	4.107	[-42;-19;56]
				<0.001	4.085	[-36;-28;65]
				<0.001	3.957	[-33;-25;53]
		0.983	81	0.001	3.795	[-39;-7;47]
		0.997	27	0.001	3.606	[-33;-22;47]
	<i>SMA</i>	<0.001	5481	<0.001	7.087	[-3;-4;65]
				<0.001	6.178	[-12;-4;74]
				<0.001	5.345	[-9;-1;53]
	<i>Thalamus</i>	0.505	513	<0.001	4.352	[-18;-16;14]
				<0.001	4.153	[-9;-22;5]
	<i>Cerebellum</i>	0.003	3024	<0.001	8.799	[12;-55;-25]
				<0.001	7.671	[18;-49;-19]
				<0.001	5.159	[24;-43;-31]
		0.001	3915	<0.001	7.285	[24;-58;-46]
				<0.001	7.255	[12;-73;-46]
				<0.001	6.467	[6;-67;-31]

Results from the random effect group analyses on the brain activation during thumb movements to command in the baseline run for the first session. Results survived a threshold of uncorrected $p < 0.001$. We highlight in bold the coordinates that we subsequently used as a starting point to search for individual coordinates to extract time series

for the DCM. Abbreviations: FWE, family wise error; MNI, Montreal Neurological Institute; M1, primary motor cortex; SMA, supplementary motor area.

Supplementary Information

Supplementary Table 1. Effects of tDCS on behavioural metrics

Metrics	Polarity	Baseline	Post-tDCS	F	p
Experiment 1 - M1-tDCS					
Reaction time (<i>ms</i>)	Anodal	0.29 (± 0.06)	0.27 (± 0.05)	1.312	0.283
	Cathodal	0.27 (± 0.05)	0.26 (± 0.04)		
	Sham	0.28 (± 0.05)	0.28 (± 0.07)		
Mean Velocity (<i>cm/s</i>)	Anodal	8.39 (± 3.98)	7.59 (± 2.81)	1.939	0.160
	Cathodal	7.71 (± 4.49)	7.26 (± 3.83)		
	Sham	6.96 (± 3.14)	6.99 (± 2.83)		
Peak acceleration (<i>m/s²</i>)	Anodal	31.05 (± 41.64)	37.31 (± 56.13)	0.636	0.536
	Cathodal	32.81 (± 38.32)	24.11(± 29.34)		
	Sham	77.82 (± 177.01)	111.39 (± 244.91)		
Experiment 2 - cb-tDCS					
Reaction time (<i>ms</i>)	Anodal	0.30 (± 0.04)	0.28 (± 0.04)	0.951	0.395
	Cathodal	0.29 (±0.03)	0.28 (± 0.04)		
	Sham	0.30 (± 0.05)	0.29 (±0.05)		
Mean Velocity (<i>cm/s</i>)	Anodal	7.44 (± 3.53)	7.78 (± 3.98)	0.951	0.395
	Cathodal	7.11 (± 3.18)	6.38 (± 2.77)		
	Sham	8.09 (± 2.73)	8.09 (± 2.55)		
Peak	Anodal	39.29 (± 31.94)	39.29 (± 33.62)	0.404	0.670

acceleration (m/s^2)	Cathodal	51.19 ($\pm$ 96.90)	31.74 ($\pm$ 15.27)		
	Sham	77.82 ($\pm$ 141.91)	60.96 ($\pm$ 108.44)		

Statistics for the interaction between polarity (anodal, cathodal, sham) and run (baseline vs post-tDCS) on average reaction time, mean velocity and peak acceleration for Experiment 1 and Experiment 2.

Abbreviations: ms, milliseconds; cm, centimetres; s, seconds; m, metres.

Supplementary Table 2. Blinding

Experiment	Active Stimulation	Sham	χ^2	p
Experiment 1	31/43	15/22	0.9682	0.701
Experiment 2	33/40	13/22	0.0869	0.263

Number of times that each type of stimulation was perceived as real, and statistics for the corresponding McNemar's Test. Active stimulation includes anodal and cathodal sessions.