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Multi-session tDCS paired with passive mobilisation of the thumb modulates thalamo-cortical coupling during command following in the healthy brain

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A B S T R A C T

Therapeutic options to restore responsiveness in patients with prolonged disorder of consciousness (PDOC) are limited. We have recently shown that a single session of tDCS over M1 *delivered at rest* can reduce thalamic self-inhibition during motor command following. Here, we build upon this by exploring whether pairing tDCS with a concurrent passive mobilisation protocol can further influence thalamo-M1 dynamics and whether these changes are enhanced after multiple stimulation sessions. Specifically, we used Dynamic Causal Modelling (DCM) of functional magnetic resonance imaging (fMRI) data from 22 healthy participants to assess changes in effective connectivity within the motor network during active thumb movements after 1 or 5 sessions of tDCS paired with passive mobilisations of the thumb. We found that a single anodal tDCS session decreased self-inhibition in M1, with five sessions further enhancing this effect. In addition, anodal tDCS increased thalamo-M1 excitation as compared to cathodal stimulation, with the effects maintained after 5 sessions. Together, our results suggest that pairing anodal tDCS with passive mobilisation across multiple sessions may facilitate thalamo-cortical dynamics that are relevant for behavioural responsiveness in PDOC. More broadly, they offer a mechanistic window into the neural underpinnings of the cumulative effects of multi-session tDCS.

1. Introduction

Prolonged disorders of consciousness (PDOC), including the vegetative/unresponsive wakefulness state (VS) and the minimally conscious state (MCS) are clinically expressed by reduced, or absent, behavioural responsiveness, and this forms the basis of their diagnosis (Royal College of Physicians, 2013). For most patients, their lack of responsiveness is a true reflection of reduced or absent awareness. However, in a significant minority of them, an absence of command-following masks a high level of cognitive functioning and awareness which cannot be captured clinically, and responds to a deficit of voluntary motor control instead (Fernandez-Espejo and Owen, 2013; Schiff, 2015). This condition is known as cognitive-motor dissociation (CMD) (Schiff, 2015) and is associated with reduced thalamo-cortical coupling owing to selective structural impairments in the pathways connecting the thalamus and primary motor cortex (M1) (Fernandez-Espejo et al., 2015; Stafford et al., 2019). In an earlier study (Aloi et al., 2022), we used fMRI to demonstrate that a single session of tDCS over M1 delivered at rest can successfully modulate thalamo-cortical dynamics during a subsequent behavioural command-following task in the healthy brain (Aloi et al., 2022), suggesting a potential route to improve responsiveness in PDOC. This added to existing promising results from clinical trials (see Aloi et al., 2021 for a review) but switched the focus from the consciousness disorder itself to a mechanistic target based on the patients' lack of ability to produce voluntary motor responses.

While our earlier results are encouraging, it is generally accepted that tDCS leads to most reliable behavioural effects when paired with a task that can successfully engage the target network (Kadosh et al., 2010; Galea and Celnik, 2009; Koyama et al., 2015; Li et al., 2019). In fact, it is thought that tDCS reinforces the pattern of underlying brain network activity (Abellaneda-Pérez et al., 2020; Dubreuil-Vall et al., 2019; Li et al., 2019), by selectively modulating the neurons that are active at the time of stimulation (Wang et al., 2023). Therefore, its effects on brain function are dependent on the ongoing neuronal state and are maximised when participants are able to actively engage in a relevant task. Similarly, work in stroke and other clinical conditions affecting motor function has shown that concurrent stimulation and physical therapy help promote motor rehabilitation, as tDCS appears to enhance the response of neural networks to the therapy, optimising plastic changes as a result (Bolognini et al., 2009; Ehsani et al., 2022; Jin et al., 2019; Morrison-Ham et al., 2022). Crucially, unresponsive patients who

Abbreviations: PDOC, Prolongued Disorders of Consciousness; fMRI, functional Magnetic Resonance Imaging; DCM, Dynamic Causal Modelling; tDCS, Transcranial Direct Current Stimulation.

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cannot engage in active rehabilitation are currently limited to receiving tDCS at rest (see e.g., (Aloi et al., 2021). Here, we aim to explore whether passive mobilisation can provide an alternative option to the use of an active, voluntary task to maximise tDCS effects. Indeed, the literature suggests that passive mobilisation may lead to an engagement of the motor areas (Alary et al., 1998; Boscolo Galazzo et al., 2014; Jaeger et al., 2014; Lotze et al., 2003; Zhavoronkova et al., 2017) and modulations of cortical excitability (Chye et al., 2010; Miyaguchi et al., 2013; Sasaki et al., 2018) similar to active movements. Moreover, TMS studies have shown that peripheral sensory input arising from passive mobilisations does reach the motor cortex and influences subsequent production of active movements (Lewis et al., 2001). To our knowledge, only 2 studies (Koh et al., 2017; Ochi et al., 2013) have combined passive mobilisations with tDCS before. These focused on post-stroke rehabilitation and found modest effects. Little is known about the mechanisms of action underlying any potential potentiation of tDCS effects due to passive mobilisation.

In addition to task-pairing, it is well-known that, while a single session of tDCS can improve motor performance, it may not be sufficient to induce long-lasting behavioural (Hashemirad et al., 2016) or clinical (Navarro-López et al., 2022; Yosephi et al., 2018) changes. In contrast, multiple sessions can lead to more clinically meaningful improvements that are sustained over weeks or months (Bruce et al., 2020; Kim et al., 2019; Lefaucheur et al., 2017; Navarro-López et al., 2022). This is possibly due to plastic changes in synaptic efficacy that may translate into long-lasting functional gains (Bolognini et al., 2009). In the context of motor learning, this has been related to a potentiation of the functional connectivity in relevant networks (Floyer-Lea and Matthews, 2005). Importantly, most studies focus only on single- or multiple-session protocols and direct comparisons between them are scarce (Hashemirad et al., 2016; Navarro-López et al., 2022). Moreover, the underlaying neural bases of incremental multi-session effects of stimulation are poorly understood.

In this study, we used dynamic causal modelling of fMRI data to explore single- and multi-session effects of tDCS over M1, paired with passive mobilisation of the thumb, on thalamo-cortical coupling during command-following. We focused on characterising the mechanisms and effects of tDCS on a well-controlled study in healthy individuals, before translating our protocols to clinical groups. We hypothesised that: (a) the combination of neurostimulation and passive mobilisation will enhance thalamo-cortical coupling beyond what we have previously reported when administering stimulation at rest (Aloi et al., 2022), and (b) such enhancement will be greater after multiple stimulation-mobilisation sessions.

2. Materials and methods

2.1. Participants

Twenty-four right-handed healthy volunteers took part in the study (16 women, 8 men; mean age 24 ± 3 years), of whom 22 completed all three weeks of testing and were therefore included in the analyses (16 women, 6 men; mean age 25 ± 4 years). We recruited participants using advertisements across campus as well as the local Research Participation Scheme. All participants completed a pre-screening protocol to ensure eligibility to safely participate in MRI and tDCS experiments. They all reported no history of psychiatric or neurological disorders, no personal or family history of epilepsy, no use of psychoactive drugs, and normal or corrected vision. We instructed participants to come to the sessions well rested and hydrated, and not to consume coffee or alcohol within the 24 h before each session, as per tDCS safety regulations (Antal et al., 2017). All participants completed the Edinburgh handedness inventory (Oldfield, 1971). The University of Birmingham's Science, Technology, Engineering, and Mathematics Ethical Review Committee approved the study, and all participants gave written informed consent. We compensated participants with £220 or the equivalent in course credits.

2.2. Experimental procedure

We used a sham- and polarity-controlled, randomised, blind, crossover design. Our protocol involved 3 testing weeks in which participants received 5 consecutive daily sessions of either anodal, cathodal, or sham tDCS, in a counterbalanced order (i.e., one polarity per week, see Fig. 1A). There was at least 1 week break between each polarity (mean days: 9 ± 15). Participants were blind to the polarity used in each session. Each week, we delivered the first and fifth sessions of stimulation in the MRI scanner (we will refer to these as Day1-MRI and Day5-MRI respectively), where participants also performed a command following task (CF-fMRI) before and after the stimulation (this resulted in 12 fMRI runs of the task per participant). We delivered the second, third, and fourth tDCS sessions in a designated cubicle (we will refer to these as Day2-Lab, Day3-Lab, and Day4-Lab). We screened participants to reconfirm MRI safety before each MRI session and to comply with tDCS safety requirements before every stimulation session.

We used an MRI-compatible joystick (FORP-190 932, Current designs INC., PA USA) to record participants' responses during CF-fMRI (1200 Hz sampling frequency of x and y positions) and to deliver the passive mobilisation. For the MRI sessions, we placed the joystick on the participant's torso and secured their right thumb to the handle using tape. During the lab sessions we secured the joystick on a desk while the participant was sitting on a chair. In the MRI sessions, participants completed the CF-fMRI task before and after tDCS (paired with passive mobilisations). In the lab sessions, participants only received the stimulation/mobilisations but did not have to perform the task.

We used a Windows 10 Desktop PC and Matlab 2017b to deliver the stimuli and record the motion tracking data. In the MRI sessions, we presented all visual stimuli via a VPixx PROPIxx system that projected the image onto a mirror fixed to the head coil, with a visual angle of ~10° We delivered auditory cues using the SOUNDPixx MRI stereo audio system coupled with disposable noise-attenuating (about 29db+) ear tips (MRIaudio INC). In the lab sessions we used an Iiyama 24" desktop monitor and provided participants with both earplugs and 3 M ear defenders (35 dB).

After all sessions, participants received a post-tDCS perceptual scale where they rated the sensations and/or discomfort they perceived and indicated whether they thought they received real stimulation or sham.

2.3. Electrical stimulation

To administer the stimulation, we used a NeuroConn DC-STIMULATOR-MR for the MRI sessions and a DC-STIMULATOR for the lab sessions (neuroCare Group GmbH, Germany). We used 5 × 5 cm2 electrodes with a thin layer of electro-conductive Ten20® paste, secured on the scalp using self-adhesive tape. We placed the target electrode over the left M1 and the reference electrode over the right orbitofrontal area (see Fig. 1B for a graphical representation of the montage and simulation of the current spread), using a standard 10–20 system EEG cap to mark the position of C3 and Fp2. Note that we use the terms 'target' and 'reference' per convention and for consistency with our own previous work (Aloi et al., 2022; Calzolari et al., 2023) while acknowledging that both electrodes are indeed active (see Fig. 1B). For anodal stimulation we placed the anode over C3 and the cathode over Fp2 and we reversed this for cathodal sessions. We used the anodal montage in half of the sham sessions, and the cathodal montage in the other half.

In anodal and cathodal sessions, we stimulated for 20 min at 1 mA, with 30s ramp-up and 30s ramp-down. Sham stimulation lasted only 30s - also with 30s ramp-up and 30 s ramp-down - to give the feeling of active stimulation, in line with well-established protocols used to ensure blinding (Woods et al., 2016).

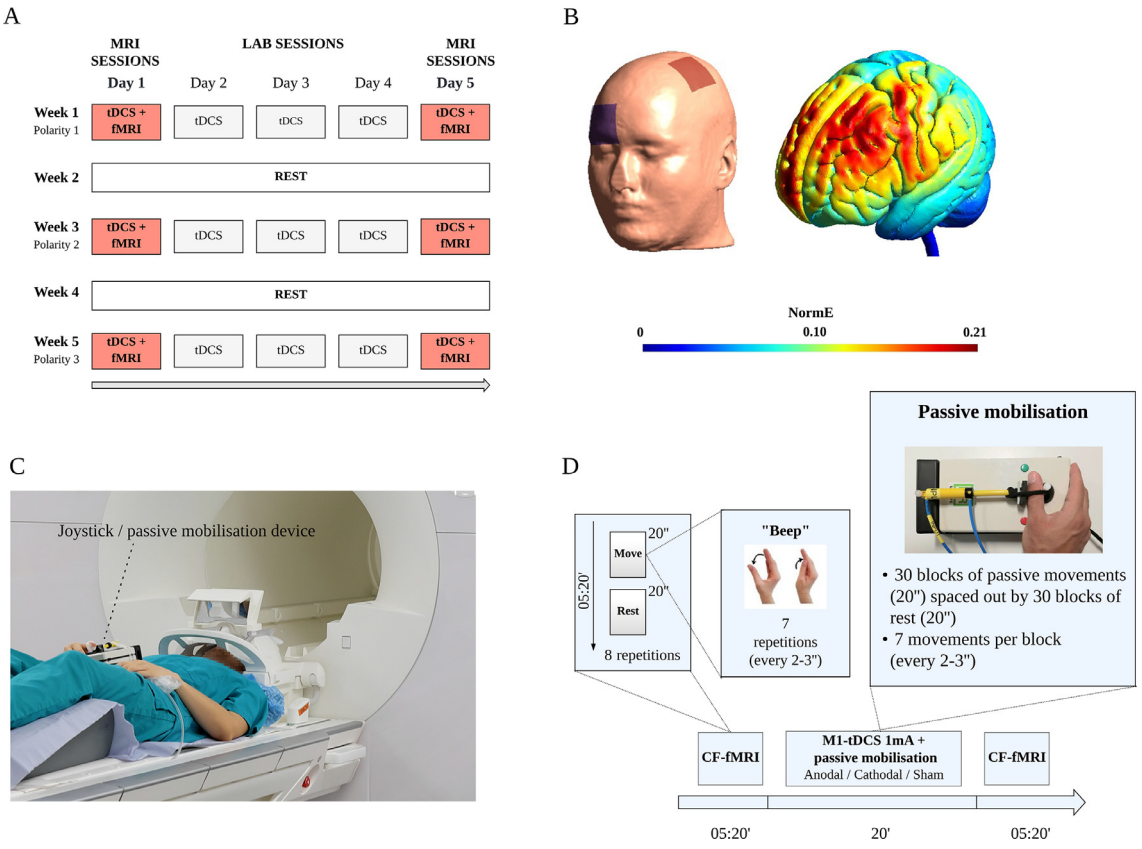

Fig. 1. Study Design and tDCS montage. (A) Schedule of events for all participants. Note that tDCS was coupled with passive mobilisation of the thumb in all sessions. (B) tDCS montage (left) and simulation of current spread (electric field) on the MNI standard head model (right), as calculated with SimNIBS3.2.2. We placed the target electrode on C3 (M1) and the reference electrode on Fp2 for the simulation and set up the current to 1 mA. Note that this simulation does not consider inter-individual differences in the position of the electrodes or the different tissue compartments across participants and therefore it should be interpreted as a rough estimate of the canonical field spread that is expected with our montage. (C) MRI setup. We recorded participant movements during the command-following (CF-fMRI) task with an MRI compatible joystick, which we also used to deliver passive mobilisation during tDCS. (D) MRI session. Participants performed the CF-fMRI task before and after receiving 20 min of tDCS coupled with passive mobilisation of the thumb.

2.4. MRI acquisition

We acquired all MRI data on a Siemens MAGNETOM Prisma 3T system, with a 64-channel head coil, at the Centre for Human Brain Health (University of Birmingham), using the following fMRI parameters: 63 slices, TR = 1620 ms, TE = 35 ms, matrix size = 84 × 84 × 63, voxel size = 2.5 × 2.5 × 2.5, no gap, flip angle = 71° and iPAT acceleration factor = 3, 198 vol per CF-fMRI run.

In addition to the functional scans, we also acquired a high-resolution T1-weighted MPRAGE image, used for anatomical co-registration, using the following parameters: TR = 2000 ms, TE = 2.03 ms, matrix size = 256 × 256 × 208, voxel size = 1 × 1 × 1 mm, and flip angle = 8°.

We also collected diffusion tensor imaging data, fMRI data during stimulation, and resting state fMRI before and after the stimulation. However, we will analyse and report these in separate papers.

2.5. fMRI paradigm

We used the same paradigm described in (Aloi et al., 2022), whereby we instructed participants to perform a simple thumb movement (adduction-abduction), as fast as possible, in response to an auditory cue (beep) (Fig. 1C and 1D). Beeps appeared in 8 blocks of 20s (cued with the word "move") and alternated with rest blocks (cued by the word "relax"), for a total duration of 5 min and 20s. Each active block included 7 beeps, which appeared at a variable interstimulus time, ranging from 2 to 3s, to avoid prediction effects. At the beginning of each run of the task, we displayed the following written instructions: "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".

2.6. Passive mobilisation

In all sessions (MRI and Lab), we passively moved the participant's right thumb during the 20 min of tDCS stimulation. For this, we used a custom-made system that involved a piston attached to the FORP joystick (Fig. 1D). The piston was connected to an air compressor through plastic tubes. We used a Jun-Air Compressor Sj-27 placed in the MRI control room during the MRI sessions, and a Bambi BB24 air compressor (24 litres) in the lab sessions. To open and close the airflow and move the piston, we used an electrically-operated valve, controlled with a Matlab script. In order to achieve a full range of movement (i.e., full abduction-adduction of the thumb), we secured the piston to the participant's thumb using a rubber band. This set-up was the same for both MRI and lab sessions. We delivered passive movements in blocks of 20s, with 7 movements per block (with a variable time interval of 2–3s), and 20s rest between blocks, to emulate the timings in the command-following task. The total number of passive movements administered during the 20 min of stimulation was 210 (i.e., 30 blocks of passive movements and 30 blocks of rest). Importantly, we fitted participants with noise attenuating earphones both in the MRI and the lab sessions, to prevent them from hearing any noise produced by the air-compressor and piston, and being able to anticipate the passive movements as a re-

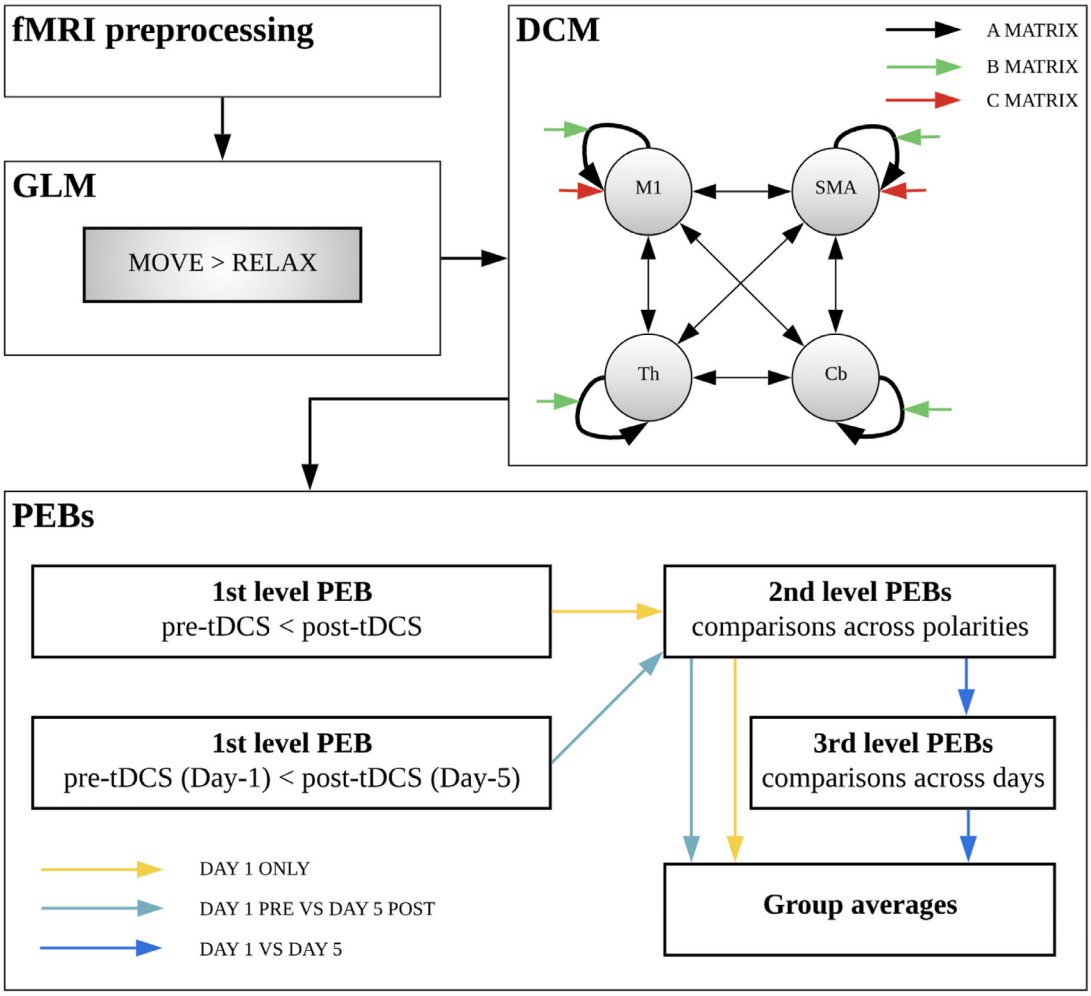

Fig. 2. Analysis pipeline. We followed a standard pre-processing protocol (not displayed), followed by general linear model analyses to obtain single-subject and group activation across all runs (*GLM* - panel). We then built and estimated DCMs for each participant and run (see *DCM* panel for a description of our model space). Finally, we used Parametric Empirical Bayes (PEB) to test group differences in effective connectivity (*PEB* panel). Abbreviations: GLM, general linear model; DCM, dynamic causal modelling; M1, primary motor cortex; SMA, supplementary motor area; TH, thalamus; CB, cerebellum; PEB, parametric empirical bayes.

sult (see experimental procedure). Note that the scanner noise provided further masking. We also instructed participants to keep their gaze at a fixation cross throughout. Additionally, in the MRI scanner participants were laying in a supine position and could not see their thumb moving, ensuring no visual feedback.

2.7. fMRI preprocessing and DCM analysis of the command-following task

2.7.1. fMRI preprocessing

We used SPM12 on MATLAB 2015b for the preprocessing of the fMRI data and followed a standard pipeline, similar to our earlier study (Aloi et al., 2022). This included realignment, co-registration between the structural and functional scans, spatial normalisation, and smoothing with an 8 mm FWHM Gaussian Kernel.

2.7.2. Region selection and time series extraction

We followed a similar pipeline to our previous study (Aloi et al., 2022): First, we identified the canonical pattern of activity on our command-following task at the group level (Fig. 2, green box). To this end, we first built a multi-session 1st-level fixed-effects (FX) model for each individual subject, including all 12 command-following task runs (pre and post stimulation x Day1/5 sessions x 3 testing weeks) and modelling the contrast corresponding to the move vs rest blocks across sessions (non-weighted average). Then, we performed a second-level random-effects (RX) 1-sample *t*-test on the individual contrasts to assess the group effects. Specifically, we conducted region of interest (ROI) analyses on the following ROIs obtained from the AAL atlas as implemented on SPM (Tzourio-Mazoyer et al., 2002): left precentral gyrus (M1), left supplementary motor area (SMA), left thalamus (TH), and right cerebellar lobes IV-V and VIII (CB). For each ROI, we thresholded results using a family-wise error (FWE) corrected $p<0.05$ and identified the peak of activation (see coordinates in bold in Table 1). These group-derived coordinates then acted as a starting point for identifying, in each individual run, the nearest local maxima. We constrained the search of the individual peaks within a fixed sphere centred on the group coordinates and with a size of 13 mm radius for the left SMA, M1 and right cerebellum, and 7 mm for the left thalamus. The difference in the allowed distance from the group coordinates accommodated for the differences in the regions' anatomical sizes. Within that sphere, we automatically searched for individual peaks exceeding a liberal statistical threshold of uncorrected $p<0.05$ (Zeidman et al., 2019a). When no peak was found for a specific run at $p<0.05$, we iteratively relaxed the threshold in 0.05 increments until reaching $p = 0.25$. When no peak could be found at this threshold, we used the group coordinates instead (Zeidman et al., 2019b). It is important to mention that we only used these thresholds to identify the individual peak coordinates used for the feature selection (i.e., the extraction of the time series) but not for any statistical analyses. Once we identified the individual peak coordinates

for each run, we extracted time series from 4 mm radius spherical volumes of interest centred on them.

2.7.3. Individual level DCM specification and definition of model space

After the extraction of the time series, we specified dynamic causal models (Fig.2, DCM panel) at the individual level with a deterministic model for BOLD signal and the following parameters: one state per region, bilinear modulatory effects, and mean-centred inputs.

We defined our model space using the same parameters to those in our earlier study (Aloi et al., 2022): a fully connected A matrix (i.e., with all self- and between region connections switched on) including left M1, left SMA, left thalamus and right cerebellum; a B matrix including the effect of the motor task as modulatory input on all self-connections; and a C matrix modelling driving inputs to cortical regions only (M1 and SMA).

2.7.4. tDCS effects on effective connectivity

With the above DCMs, we employed Parametric Empirical Bayes (PEB) to remove the parameters that were not contributing to the model evidence, and to evaluate group effects and between-subject variability (Zeidman et al., 2019b). In order to test the effects of tDCS combined with passive mobilisation on the connections in our model, we ran three separate hierarchical PEB analyses (Zeidman et al., 2019b), starting from the 12 neural models (DCMs) estimated per participant (i.e., one per CF-fMRI run).

(1) To assess the effects of a single tDCS session, we first built 3 *within-subject* PEBs (1st-level) per participant encoding the differences between pre- and post- stimulation for each polarity on the first MRI session. We then entered these into a further 3 PEBs (2nd level) per participant that each encoded a pair-wise comparisons between polarities on the differences between pre- and post tDCS. Finally, we specified 3 group (*between-subject*) PEBs encoding the commonalities across participants for each 2nd-level PEB, and included sex, age, and handedness score as regressors of non-interest (3rd level). The 3 final PEBs therefore encoded increases (i.e., pre-tDCS<post-tDCS) in effective connectivity that are greater after anodal tDCS as compared to sham (i.e., anodal > sham), after anodal tDCS as compared to cathodal tDCS, or after cathodal tDCS as compared to sham respectively.
(2) To assess the cumulative effects of 5 tDCS sessions, we replicated the above steps but encoding the differences between the pre-tDCS run in Day1-MRI and the post-tDCS run in Day5-MRI for the 1st level of the hierarchy. 2nd and 3rd level PEBs were as above.
(3) Finally, to establish whether the responsiveness to tDCS increases after multiple sessions, we replicated the 1st and 2nd level steps in (1) above for the data on Day5-MRI. Then created a further *within-subject* PEB modelling the differences between sessions (Day1 < Day5) for each individual participant (3rd level). Finally, a between subjects PEB modelled group effects (mean across participants), and the above mentioned regressors of no interest (4th level).

Finally, we applied Bayesian Model Reduction (BMR) on the final PEBs for each analyses above (i.e., those encoding group commonalities), to reduce connections that were not adding to the model evidence, and used Bayesian Model Average (BMA) to estimate the average parameters across participants for each connection that remained switched on. We used a threshold of a posterior probability > 95%, as we have previously done (Aloi et al., 2022).

In addition, to assess the stability of our parameters across sessions, we created 3 PEBS modelling the average across sessions on the baseline pre-tDCS run on Day 1 for each polarity (i.e., run unaffected by any stimulation or passive mobilisation) and used BMR and BMA to estimate parameters as above.

2.8. Motion tracking

We analysed the motion data for the CF-fMRI task with a custom MATLAB 2017b script, as we did in our previous study (Aloi et al., 2022). We first low-pass filtered the data at 15 Hz, calculated the Euclidean distance of the x-y position, and used the MATLAB function *findchangepts* to identify the onset and the end of each movement. When more than two changes were identified, we used the first and last ones to determine the beginning and end of the movement. We excluded movements where no change was detected, which was usually due to participants not responding to that specific stimulus (on average we removed <1 movement per session, maximum of 12). For each movement we calculated velocity and acceleration at each timepoint and extracted mean velocity and peak acceleration for the whole run. We also calculated mean reaction times, defined as the time interval between the auditory stimulus and onset of the movement, identified as per above. We excluded measurement errors, defined as values with a z-score > 3.0, that were present due to the joystick being incorrectly calibrated. Out of 264 runs (per analysis), we removed 14 from the reaction times analysis, 1 from the mean velocity analysis and 36 from the peak acceleration analysis. Finally, for each of the three metrics (i.e., reaction time, mean velocity and peak acceleration) we computed three Bayesian repeated measures ANOVAs on JASP, v. 0.16.3 (JASP Team, 2020) to emulate the DCM analyses above:

(1) A Polarity (anodal, cathodal and sham) x Time (pre- vs post-tDCS) ANOVA on the data from Day1-MRI tested the effects of a single tDCS session.
(2) A Polarity x Multiple session time (Day1-MRI pre-tDCS vs Day5-MRI post-tDCS) ANOVA tested the effects of 5 sessions.
(3) A Polarity x Time x Day (Day1-MRI vs Day5-MRI) ANOVA tested whether within-session responsiveness to tDCS is affected by the number of sessions.

For each ANOVA, we calculated the evidence in the data for including the interaction (e.g., polarity x time) as a predictor (BF_{incl}); that is, whether the models including the interaction explain the data significantly better than those without it (van den Bergh et al., 2020). We interpreted $BF_{incl} > 3$ as substantial evidence in the data for including the interaction, $BF_{incl} > 10$ as strong evidence, and $BF_{incl} > 100$ as very strong evidence, as per standard guidelines on interpreting Bayes factors (Schönbrodt and Wagenmakers, 2018). Importantly, for $BF_{incl} < 3$, we reported the exclusion Bayes factor (BF_{excl}) instead, which quantifies the opposite effect (the evidence for models excluding the interaction as compared to those including it).

2.9. Blinding

To test the success of our blinding protocol, we used McNemar's test on participants' responses as to whether they believed they had / had not received real tDCS on days 1 and 5 (MRI sessions).

3. Results

3.1. Task activation across sessions

The RFX second-level analysis of brain activation during the active movements to command across sessions showed statistically significant clusters (FWE-corrected $p<0.05$) in all of four ROIs considered in our analyses (see Table 1 and Fig. 3).

3.2. Effects of M1-tDCS on motor-network dynamics

3.2.1. Effects of one M1-tDCS session (Day1-MRI)

A single anodal M1-tDCS session (Fig. 4, panel A) increased excitation from M1 to SMA, when compared to both cathodal- and sham-tDCS, and from thalamus to M1 as compared to cathodal tDCS only.

Table 1
Canonical activation during command-following.

Region	Cluster P	Cluster size	Peak P		F	MNI coordinates
	FWE-corrected	*in mm3*	*Peak FWE-corrected*	*uncorrected*		*[x;y;z]*
Cerebellum	<0.001	3969	<0.001	<0.001	11.871	**[12;−46;−19]**
			<0.001	<0.001	11.337	[9;−52;−13]
	<0.001	729	<0.001	<0.001	9.465	[12;−58;−43]
	<0.001	162	0.005	<0.001	7.399	[27;−49;−46]
M1	<0.001	5697	<0.001	<0.001	10.570	**[−36;−19;56]**
			<0.001	<0.001	9.695	[−27;−19;74]
			<0.001	<0.001	9.174	[−33;−13;65]
	0.026	27	0.008	<0.001	7.126	[−42;−1;20]
SMA	<0.001	3834	<0.001	<0.001	9.812	**[−6;−1;56]**
			0.004	<0.001	7.450	[−9;8;44]
Thalamus	0.002	297	<0.001	<0.001	9.506	**[−18;−22;11]**

Results from the random effect group analyses (RFX, 1-sample *t*-test) for the command-following task, including all 12 MRI sessions per participant. Results survived a threshold of FWE-corrected $p < 0.05$. We used the peak coordinates for each region (highlighted in bold) as a starting point to extract the time series for the DCM analyses. Abbreviations: FWE, family wise error; M1, primary motor cortex; SMA, supplementary motor area.

Additionally, when compared to sham only, anodal tDCS decreased M1 self-inhibition as well as excitation from SMA to thalamus. In turn, a single session of cathodal tDCS, increased cerebellar self-inhibition, when compared to both anodal and sham, and thalamic self-inhibition when compared to sham only. Both anodal and cathodal tDCS increased excitation from SMA to M1 and inhibition from cerebellum to M1, although these changes were more pronounced for cathodal and anodal tDCS respectively. Similarly, both polarities increased inhibition from thalamus to SMA and cerebellum, as well as from cerebellum to SMA, with no differences between polarities in this case. In terms of task modulations on each self-connection (matrix B), we found no significant effects for any polarity.

See Fig. S1 for baseline (pre-tDCS) connectivity values (Fig. 5).

3.2.2. Effects of multiple M1-tDCS sessions (Day1-MRI-pre vs day5-MRI-post)

After 5 sessions, anodal tDCS increased excitation from thalamus to M1 when compared to cathodal tDCS only. It also increased self-inhibition in SMA and reduced self-inhibition in the cerebellum as compared to sham only. Finally, it increased excitation from cerebellum to M1 when compared to both cathodal and sham. Both anodal and cathodal tDCS increased excitation from thalamus to SMA, although this was significantly stronger for anodal stimulation. Similarly, both polarities reduced M1 self-inhibition and excitation from thalamus to cerebellum compared to sham, with no significant difference between polarities.

In turn, cathodal tDCS increased excitation between M1 and SMA (in both directions) and inhibition from cerebellum to thalamus and SMA, both as compared to anodal tDCS and sham. As above, we found no significant effects in task modulations for either polarity.

3.2.3. Incremental within-session effects after five M1-tDCS sessions (Day1-MRI vs day5-MRI)

Compared to the first session, the fifth session of anodal M1-tDCS (Fig. 4, panel B) reduced M1 self-inhibition as compared to sham only. In addition, both polarities increased excitation from thalamus to SMA and from cerebellum to M1, and reduced inhibition from thalamus to cerebellum and cerebellum to SMA, with no differences across polarities.

In turn, the fifth session of cathodal tDCS reduced thalamo-M1 inhibition, and decreased cerebellar self-inhibition and excitation from SMA to M1. All of these were polarity specific, i.e., significant both when compared to anodal tDCS and sham. As above, we found no significant effects in task modulations for either polarity.

3.3. Effects of M1-tDCS on behavioural metrics

We found substantial evidence in support of the lack of an interaction ($BF_{excl} > 3$), for reaction times and mean velocity on Day1-MRI and Day1-MRI pre vs Day5-MRI post, as well as for peak acceleration on Day1-MRI only. In addition, we found anecdotal evidence (i.e., $BF_{excl} = 1–3$) for the lack of an interaction on mean velocity for Day1-MRI vs Day5-MRI and peak acceleration for the comparisons Day1-MRI pre vs Day5-MRI post and Day1-MRI vs Day5-MRI. We report means and standard deviations for each condition in Table S1 along with the BF_{excl} values and their respective prior and posterior inclusion probability, for all ANOVAS performed. Single-subject values are in Fig. S2.

3.4. Efficacy of blinding protocol

We found no significant differences in the number of times that participants perceived sham and anodal/cathodal stimulation as real stimulation for either Day1-MRI ($\chi^2 = 9$, $p = 0.82$) or Day1-MRI ($\chi^2 = 11$, $p = 0.20$)

4. Discussion

This study focused on characterising the effects of tDCS paired with passive mobilisation on thalamo-cortical coupling during a motor command-following task; a circuit with an established mechanistic role in the behavioural responsiveness in PDOC (Fernandez-Espejo et al., 2015). We have previously shown that one anodal M1-tDCS session administered at rest can reduce thalamic self-inhibition during command following. However, against prediction, these changes were not followed by increased excitation towards M1 (Aloi et al., 2022). Here, we demonstrated that, when paired with passive mobilisation, one session of M1-tDCS can indeed modulate thalamus to M1 excitation, (and in the case of anodal stimulation reduce M1 self-inhibition as a result), during the same command following task. While the modulations to the thalamus to M1 connection were not significant against sham, but only when the two polarities were compared, its average connectivity strength became more excitatory after anodal tDCS as compared to pre-stimulation values (see Fig. S1 and Table S2).

Interestingly, our previous study (Aloi et al., 2022) revealed unexpected effects after one session of cathodal M1-tDCS, which, against predictions, led to an increase in excitation from thalamus to M1. We attributed this to a compensatory mechanism to overcome the strong inhibition in M1 that followed this polarity and keep performance at a baseline level. In contrast, here we found that anodal and cathodal stimulation showed opposing effects over this connection (i.e., excitatory for anodal and inhibitory for cathodal), in line with our predictions and existing literature. Pairing tDCS with a relevant task is indeed regarded as best practice (Pruski and Cantarero, 2020). In fact, there is much evidence that the effect of tDCS is greatly dependent on the neural state during stimulation, and that using a task that engages the relevant neural circuit can increase the spatial precision of tDCS on the

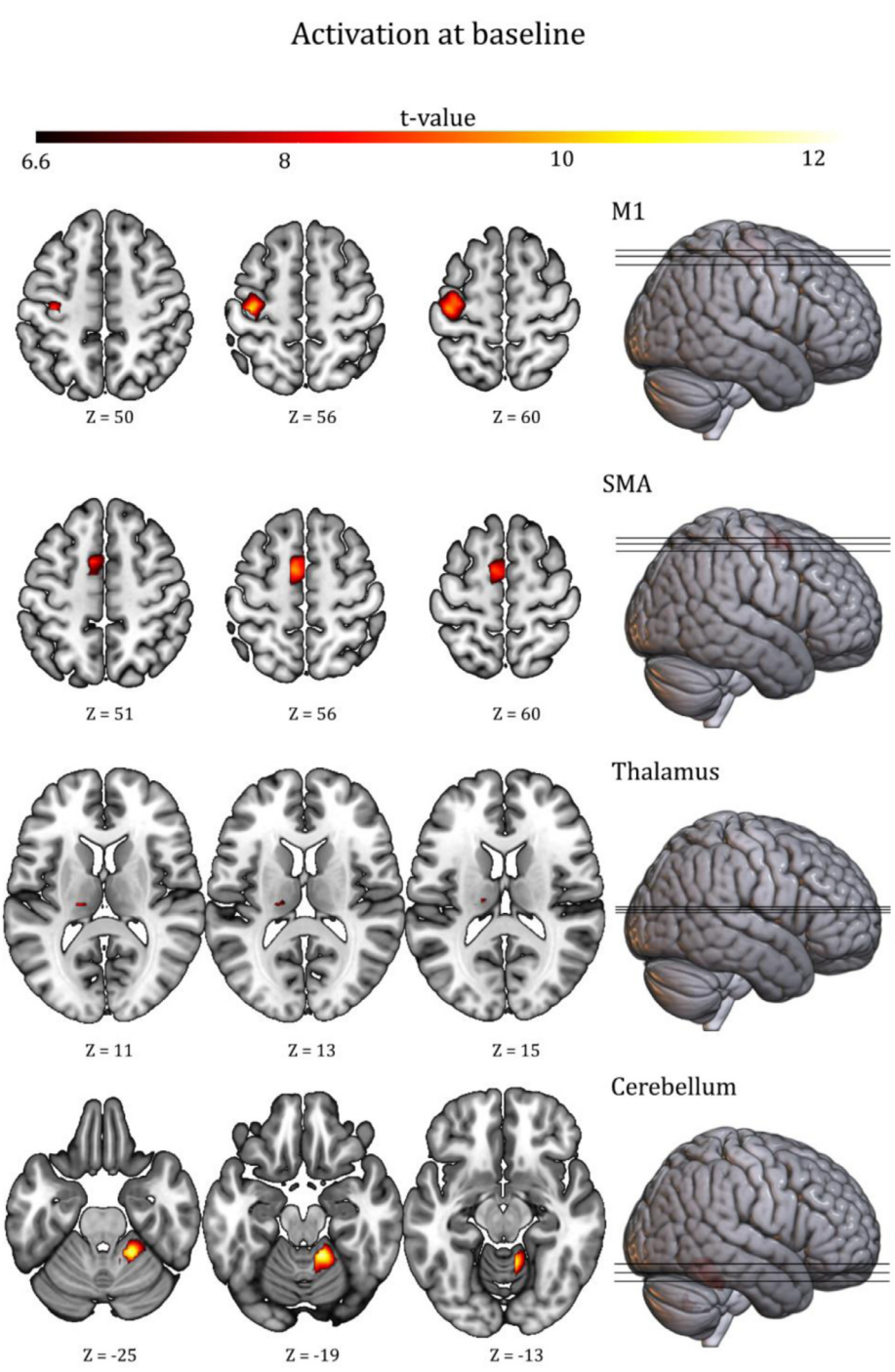

Fig. 3. Activation at baseline. Brain activation at the group level (RFX, 1-sample *t*-test) for the command-following task. The general linear model included differences between 'move' and 'rest' blocks on a multi-session 1st level (FFX) model for each subject - using all 12 MRI runs. The activation maps are shown at $p<0.05$ FWE-corrected for M1, SMA, cerebellum and thalamus ROIs, and rendered on a standard template (152 template in MRIcroGL). Z indicates the Montreal Neurological Institute z coordinate.

target networks (Li et al., 2019). Our results are in line with previous work showing that peripheral signals arising from passive mobilisations can modulate cortical excitability (Chye et al., 2010; Lewis et al., 2001; Miyaguchi et al., 2013; Sasaki et al., 2018), and further suggest that such modulations can interfere with the effects of tDCS on neural dynamics at cortical and subcortical levels.

In addition, our study offers a window into the neural underpinnings of the cumulative effects of multi-session tDCS, and revealed a somewhat complex picture, particularly for cathodal stimulation. First, after 5 tDCS sessions, anodal stimulation continued to increase thalamus to M1 excitation (albeit only significantly when compared to cathodal stimulation) and to decrease M1 inhibition as compared to baseline. Similarly, cathodal tDCS decreased thalamus to M1 excitation both after 1 and 5 sessions (although significantly less so after 5). However, after 5 sessions, cathodal tDCS also decreased M1 inhibition, and did so at a similar level to anodal tDCS. Moreover, after 5 sessions, cathodal tDCS lost the influence on increasing thalamic self-inhibition that it had after 1. Crucially, the baseline (pre-tDCS) connectivity within and between these two regions was very stable across polarity sessions (see Fig. S1 and Table S2). There were other effects outside these key areas of interest (thalamus, M1 and their connection) that only became apparent or changed tone after 5 sessions of either polarity. For example, the thalamus changed from inhibiting SMA after one session of either anodal or cathodal tDCS to exciting it after 5 sessions of either polarity. In turn, 5 sessions of anodal tDCS were required to reduce cerebellar self-inhibition, while the effects of cathodal tDCS over the cerebellum

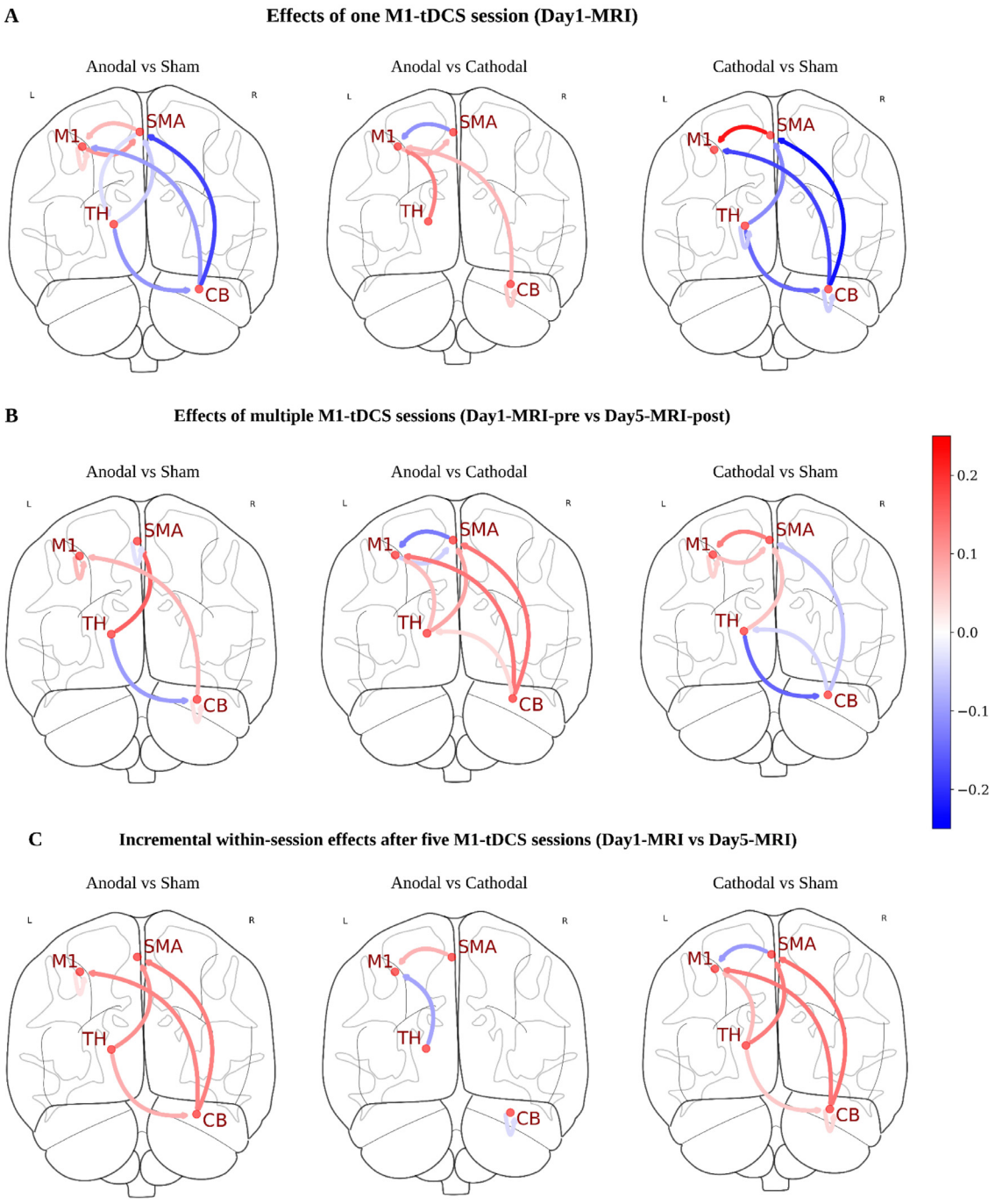

Fig. 4. Effects of M1-tDCS on neural dynamics. Results from our Parametric Empirical Bayes (PEB) analyses on Day1-MRI (A), Day1-MRI-pre vs Day5-MRI-post (B) and pre- vs post- on Day1-MRI vs Day5-MRI (C). Blue lines indicate decreases and red lines indicate increases in connectivity strength. Notice that we converted self-connections from their original unitless log-scale values to Hertz and added 0.5 to obtain the direction and magnitude of the self-connectivity change and display it in a way that is comparable with between region connections.

in session 1 disappeared after 5 sessions. Looking at the baseline connectivity, it becomes clear that the existing neural state modulates the effect of each polarity to bring the network to an optimal level that maintains behavioural performance. Overall, our results suggest that future research should not necessarily expect opposing effects for the two polarities across the network of interest, nor linear, incremental effects after multiple sessions. The comparison of within-session effects sheds further light into this (Fig. 4, panel C). For example, anodal tDCS had a stronger effect on decreasing M1 self-inhibition on the fifth session as compared to the first, suggesting a potentiation of the events (rather than a simple accumulation). In contrast, the increased excitation from thalamus to M1 related to this polarity did not exhibit significant within-session differences (i.e., it was similar in sessions 1 and 5). Finally, the inhibitory effect of cathodal tDCS over this connection was smaller in the fifth session as compared to the first.

Most of our knowledge about tDCS comes from studies focusing on local effects over M1, and much less is understood about how the modulations propagate across other regions in the motor network. It is thus not surprising that the local changes on self-inhibition we identified here are consistent with prior literature, while long-range changes

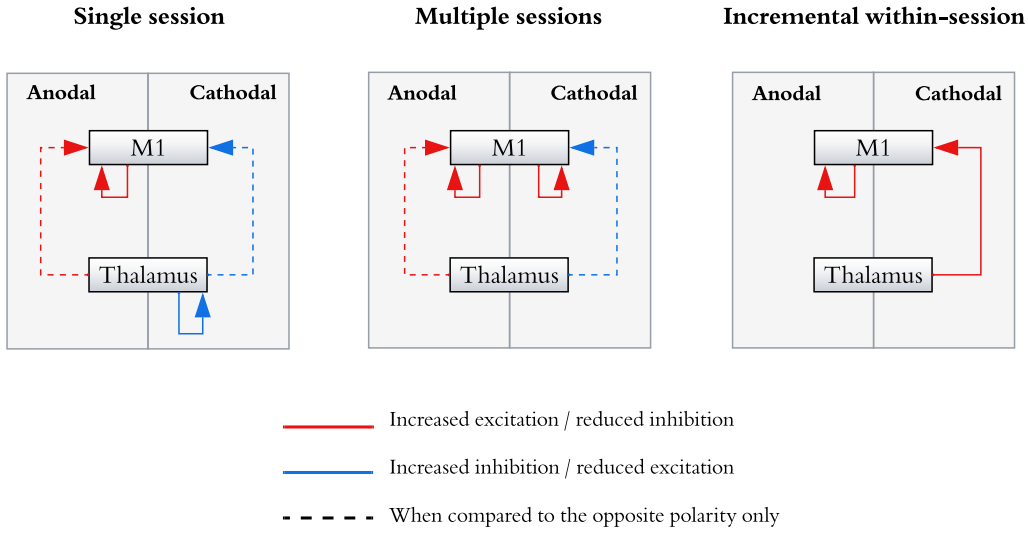

Fig. 5. Effects of M1-tDCS on thalamo-M1 coupling. Summary results for the thalamus-M1 complex after 1 session (left), 5 sessions (middle), and comparing within-session changes between day 1 and day 5 (right). Dashed lines represent effects that were only significant in the comparison between polarities (but not when each polarity was compared to sham).

appeared more complex. There is now substantial evidence that anodal tDCS over M1 increases cortical excitability (Horvath et al., 2015; Yamaguchi et al., 2020), and this modulation of neural activity is associated with changes in synaptic plasticity (Monte-Silva et al., 2013), including early- (e-LTP) and late long-term potentiation (l-LTP) when an appropriate task is used. Crucially, L-LTP requires repeated sessions of stimulation and can lead to structural plasticity that may contribute to more robust tDCS after-effects (Lu et al., 2019). Furthermore, both anodal and cathodal tDCS have a similar impact on synaptic plasticity (Lu et al., 2019), consistent with our changes after 5 sessions showing reduced M1 self-inhibition for both polarities (albeit stronger for anodal). While it is possible that e-LTP and L-LTP may be one of the mechanisms explaining the local effects on M1 in our current study, especially as we did not find an effect of anodal tDCS on M1 self-connectivity when delivering tDCS at rest in our previous study (Aloi et al., 2022), the relationship between synaptic mechanisms and effective connectivity metrics is not well understood. In fact, DCM does not model the activity of individual neurons, which are characterised by fast dynamics, but slower dynamics - in the scale of seconds - that arise from the synergy of several neuronal populations instead (Zeidman et al., 2019a). Therefore, the comparison between DCM results and neuronal models is not straightforward. Within the DCM framework, a reduction in self-inhibition reflects a lowered rate of decay of neuronal activity, which in turn indicates increased susceptibility to afferent inputs into that region (Zeidman et al., 2019a). In our case, the reported reduction in M1 self-inhibition would make M1 more sensitive to the other nodes in the network (including the excitation coming from thalamus). This way, even when the increase in excitation from thalamus to M1 seemed to be maintained (rather than enhanced) across the sessions, the enhanced responsiveness in M1 to this thalamic excitation after 5 sessions would predict a stronger clinical effect. Importantly, within our network, M1 represents the final motor output for the control of muscles, due to its projections to the spinal cord (Harrison and Murphy, 2013). As such, increasing M1 excitability via a combination of reduced M1 self-inhibition and increased thalamo-M1 excitation might successfully facilitate the initiation of movements in those PDOC patients where the cortico-thalamic tracts are partially spared.

Overall, our results suggest that pairing passive mobilisation with anodal stimulation across multiple sessions might have a therapeutic effect in PDOC, particularly in those patients whose diminished responsiveness is specifically linked to reduced thalamo-cortical connectivity (Fernandez-Espejo et al., 2015; Stafford et al., 2019). We have previously shown that *both* an excitatory engagement of the thalamus and thalamus to M1 coupling are necessary for the execution of voluntary motor responses to command in PDOC (Fernandez-Espejo et al., 2015). It is also well known that damage to the thalamus and / or its cortical projections are common in PDOC, and that these abnormalities correlate with the level of reduced responsiveness in these patients (Fernandez-Espejo et al., 2015, 2011; Schiff, 2010; Stafford et al., 2019). As individual patterns of damage are highly heterogeneous across patients, one could speculate that, depending on whether the primary damage affects mostly the thalamus or its connections to M1, different patients may benefit most from stimulation being delivered at rest (Aloi et al., 2022) or combined with passive mobilisation (as reported here) respectively, rather than one protocol being superior to the other across the whole clinical group. Further studies in PDOC patients themselves are required to ascertain this and whether the neural effects reported here indeed translate into clinically meaningful changes in this group. In addition, future studies in PDOC patients would benefit from the inclusion of fMRI assessments to characterise the neural bases of any such clinical changes and to consider the impact of each patient's neural state during stimulation on them.

There are several considerations to acknowledge. First, while our montage (C3-Fp2) is the most commonly used one to target M1 (Lefaucheur et al., 2017; Nitsche et al., 2008), the current generated is not limited to the motor cortex and reaches a widespread area instead (see Fig. 1). While the use of a motor task (both during stimulation but also to characterise its effects) can reduce the known lack of spatial specificity of tDCS (Wang et al., 2023), we cannot rule out that some of our effects may have been mediated by direct modulations of other motor areas such as SMA or the premotor cortex. Second, we used a current intensity of 1 mA to replicate the canonical excitatory / inhibitory effects of anodal / cathodal tDCS (Nitsche and Paulus, 2000) and facilitate the interpretability of or fMRI results. However, in recent years, clinical and cognitive studies have typically increased this to 2 mA (Lefaucheur et al., 2017) in an attempt to enhance the effects of stimulation. While using a higher current intensity may have resulted in greater changes to connectivity strength and / or reveal effects against sham that were only apparent when comparing between polarities, we note that the effects of tDCS are known not to follow a linear dose-response effect. Indeed, cathodal M1-tDCS switches tone from inhibition to excitation when increasing from 1 to 2 mA (Batsikadze et al., 2013). Moreover, a

recent meta-analysis suggested that intensities under 1 mA with durations over 10 min lead to greater MEPs increases than higher intensities (Dissanayaka et al., 2017). Outside the motor literature, studies have also shown that 1 mA can be more effective at inducing behavioural improvements as compared to 2 mA (Ehrhardt et al., 2021). The reasons for this are not entirely understood but some authors (Smucny, 2021) have speculated it relates to the brain's homoeostatic control of excitation / inhibition balance, whereby an overexcitation induced by higher currents may disturb such balance and lead to detrimental effects. Future research is needed to ascertain the relationship between current intensity and effective connectivity changes.

5. Conclusions

Overall, we showed that tDCS coupled with passive mobilisation of the thumb has short- and long-range effects on motor-network dynamics during command-following. Specifically, it can modulate M1 self-inhibition and excitation from thalamus to M1. Anodal effects were typically enhanced after 5 sessions of stimulation while cathodal effects varied depending on the specific node. Overall, tDCS elicited different effects than previously reported when delivering stimulation during rest, demonstrating that passive mobilisations can modulate neural responses to tDCS. While we tested our protocol on healthy participants, we designed it to be easily integrated into PDOC care as part of routine physical therapy.

Data and code availability statements

Processed data is available from the authors upon reasonable request. Please contact d.fernandez-espejo@bham.ac.uk with any questions or requests.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Credit authorship contribution statement

Davide Aloi: Investigation, Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. **Roya Jalali:** Investigation, Methodology, Writing – review & editing. **Sara Calzolari:** Methodology, Writing – review & editing. **Melanie Lafanechere:** Investigation, Writing – review & editing. **R. Chris Miall:** Methodology, Writing – review & editing. **Davinia Fernández-Espejo:** Visualization, Funding acquisition, Data curation, Methodology, Supervision, Writing – original draft, Writing – review & editing.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.neuroimage.2023.120145.

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