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Cortical activation changes underlying stimulation-induced behavioural gains in chronic stroke

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Transcranial direct current stimulation, a form of non-invasive brain stimulation, is showing increasing promise as an adjunct therapy in rehabilitation following stroke. However, although significant behavioural improvements have been reported in proof-of-principle studies, the underlying mechanisms are poorly understood. The rationale for transcranial direct current stimulation as therapy for stroke is that therapeutic stimulation paradigms increase activity in ipsilesional motor cortical areas, but this has not previously been directly tested for conventional electrode placements. This study was performed to test directly whether increases in ipsilesional cortical activation with transcranial direct current stimulation are associated with behavioural improvements in chronic stroke patients. Patients at least 6 months post-first stroke participated in a behavioural experiment (n = 13) or a functional magnetic resonance imaging experiment (n = 11), each investigating the effects of three stimulation conditions in separate sessions: anodal stimulation to the ipsilesional hemisphere; cathodal stimulation to the contralesional hemisphere; and sham stimulation. Anodal (facilitatory) stimulation to the ipsilesional hemisphere led to significant improvements (5–10%) in response times with the affected hand in both experiments. This improvement was associated with an increase in movement-related cortical activity in the stimulated primary motor cortex and functionally interconnected regions. Cathodal (inhibitory) stimulation to the contralesional hemisphere led to a functional improvement only when compared with sham stimulation. We show for the first time that the significant behavioural improvements produced by anodal stimulation to the ipsilesional hemisphere are associated with a functionally relevant increase in activity within the ipsilesional primary motor cortex in patients with a wide range of disabilities following stroke.

Keywords: transcranial direct current stimulation; stroke rehabilitation; motor system

Abbreviations: tDCS = transcranial direct current stimulation; M1 = primary motor cortex

Introduction

Chronic stroke is a leading cause of morbidity in the western world. Most patients are impaired on activities of daily living and only a small minority return to employment (Kolominsky-Rabas *et al.*, 2001; Lai *et al.*, 2002). Intensive physiotherapy remains the gold-standard treatment but outcomes are variable. Developing therapies as adjuncts to standard rehabilitation techniques to optimize functional outcome is of prime importance. Non-invasive brain stimulation has generated much interest in this context because proof-of-principle studies have demonstrated short-lived functional improvements following stimulation in chronic stroke patients (Hummel and Cohen, 2006). Transcranial direct current stimulation (tDCS) holds particular promise as the necessary equipment is comparatively inexpensive and the stimulation is easy to administer and well tolerated (Hummel and Cohen, 2006).

tDCS is targeted to the motor system by the placement of an active scalp electrode over the primary motor cortex (M1) and the reference electrode over the contralateral supraorbital ridge. Neurophysiological studies in healthy individuals have demonstrated that anodal stimulation (current flow from M1 to the reference electrode) increases cortical excitability and cathodal stimulation (current direction is reversed) decreases cortical excitability (Nitsche and Paulus, 2000).

After stroke, relatively reduced activity in ipsilesional M1 during movement of the stroke-affected hand correlates with greater functional impairments (Ward *et al.*, 2003a). This may be driven, at least in part, by abnormally high levels of inter-hemispheric inhibition from the contralesional primary motor cortex (Ward *et al.*, 2003a; Murase *et al.*, 2004). Activity within ipsilesional M1 increases over time with rehabilitation and functional recovery (Ward *et al.*, 2003b). There is therefore a strong rationale for therapies designed to facilitate activity in ipsilesional M1, either directly via facilitation of the ipsilesional hemisphere or indirectly via inhibition of the contralesional hemisphere (O'Dell *et al.*, 2009).

Behavioural studies have demonstrated that tDCS can improve motor function in chronic stroke patients for a few tens of minutes, either after anodal stimulation is applied to ipsilesional M1 (Fregni *et al.*, 2005; Hummel and Cohen, 2005; Hummel *et al.*, 2005, 2006) or cathodal stimulation to contralesional M1 (Fregni *et al.*, 2005). Daily sessions of stimulation, either anodal tDCS to ipsilesional M1 or dual stimulation, where the anode is applied to ipsilesional M1 and the cathode to contralesional M1, have been shown to improve motor function in chronic stroke for a few days (Boggio *et al.*, 2007; Lindenberg *et al.*, 2010).

However, the suggestion that benefits of either anodal stimulation applied to ipsilesional M1 or cathodal stimulation to contralesional M1 might arise by increasing ipsilesional M1 activity has not previously been tested. We aimed to test this hypothesis directly using functional MRI.

tDCS studies to date have focused on mildly impaired patients and have typically used the Jebsen Taylor Test as an outcome measure (Fregni *et al.*, 2005; Hummel and Cohen, 2005; Hummel *et al.*, 2005). This multi-part timed motor task would not be feasible for a functional MRI study and is too challenging for more impaired patients. We therefore first tested whether tDCS-evoked improvements in motor function could be detected by a simple hand motor task that could be performed by patients with a wide range of impairments both outside and inside the MRI scanner. This allowed us to relate short-term behavioural improvements evoked by tDCS with changes in brain activity. Our hypothesis was that both anodal tDCS applied to ipsilesional M1 and cathodal tDCS to contralesional M1 would lead to a decrease in response times and increase activity in the ipsilesional M1.

Patients and methods

We carried out two separate experiments, designed to assess the effects of tDCS on motor behaviour (Experiment 1) and on motor-related functional MRI activity (Experiment 2). For both experiments, patients participated in three separate sessions in randomized order at least a week apart. Different sessions were used to deliver anodal tDCS to the ipsilesional hemisphere, cathodal tDCS to the contralesional hemisphere or sham tDCS.

Patients

Patients (mean age 64 years, range 30–80 years; four female) were recruited with Local Ethical Committee approval and gave their written informed consent to participate, in accordance with the Declaration of Helsinki (Rickham, 1964). Thirteen patients participated in Experiment 1 and 11 patients in Experiment 2. Seven patients participated in both experiments, but the experiments were performed at least 1 year apart. All patients were at least 6 months post first ischaemic or haemorrhagic stroke, had no lesions in M1 and had no previous history, signs, or symptoms of other neurological conditions. No patients were on CNS-active medications. Clinical characteristics of patients studied are described in Table 1.

Transcranial direct current stimulation

A DC-Stimulator (Eldith GmbH) delivered a 1 mA current to the brain via two electrodes measuring 5 cm × 7 cm. For true stimulation, the active electrode (referred to as the M1 electrode) was centred on a position 5 cm lateral to Cz (central zero) and the reference electrode placed on the contralateral supraorbital ridge. The active electrode was placed over ipsilesional M1 for anodal stimulation, contralesional M1 for cathodal stimulation and the vertex for sham stimulation. In all cases patients were blind to the stimulation condition.

For true stimulation the current was ramped up over 10 s, held constant at 1 mA for 20 min (Experiment 1) or 10 min (Experiment 2) and then ramped down over 10 s. For sham stimulation the current was ramped up over 10 s and then immediately switched off. Subjects are not able to distinguish between true and sham stimulation using this procedure (Gandiga, 2006). For Experiment 1, saline sponges were used as a conducting medium. For Experiment 2 high chloride EEG electrode paste was used and electrodes were each fitted with 5 kΩ resistors.
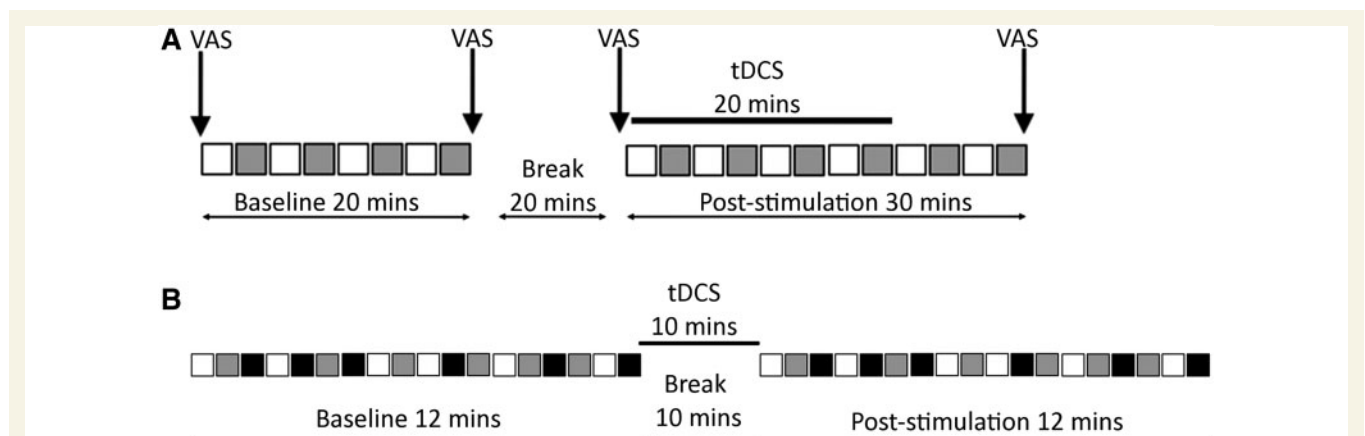
Experiment 1: Behavioural study

In all three sessions subjects were asked to perform blocks of a visually-cued response time task interleaved with a grip force task

Table 1 Patient characteristics

	Experiment 1	Experiment 2	Sex	Handedness	Age	Time since stroke (months)	Type of stroke	Lesion side	Lesion volume	Lesion location[a]	UEFM
1	+		M	Right	62	39	Infarct	Right	30	Subcortical	49
2	+		M	Right	74	18	Infarct	Right	237 084	Cortical	27
3	+		M	Right	78	22	Infarct	Left	N/A	Cortical	40
4	+		F	Right	75	35	Infarct	Left	2640	Subcortical	16
5	+		M	Right	66	64	Infarct	Left	8141	Subcortical	40
6	+		M	Right	71	34	Infarct	Left	370	Subcortical	35
7	+	+	M	Right	62	62	Infarct	Left	102 407	Cortical	57
8	+	+	M	Right	58	42	Infarct	Left	1112	Subcortical	59
9	+	+	F	Right	30	70	Haemorrhage	Right	40 398	Cortical	66
10	+	+	M	Right	80	36	Infarct	Left	671	Subcortical	63
11	+	+	F	Right	78	24	Infarct	Left	8091	Cortical	24
12	+	+	M	Right	66	35	Infarct	Right	390	Subcortical	62
13	+	+	M	Right	63	42	Infarct	Left	10 220	Cortical	24
14		+	M	Right	69	36	Infarct	Left	8820	Subcortical	51
15		+	F	Right	60	40	Infarct	Left	792	Subcortical	61
16		+	M	Right	43	18	Infarct	Left	24 732	Cortical	64
17		+	M	Right	44	28	Infarct	Left	4073	Subcortical	64

a Cortical lesions do not include the primary motor cortex.
UEFM = upper extremity Fugl-Meyer score (max score 66; higher scores reflect better motor performance).

Figure 1 Outline for experimental design for each stimulation session. (**A**) Experiment 1: Behavioural study. White blocks represent response time task, grey blocks represent grip force task. VAS = visual analogue scale; to assess fatigue, pain, discomfort and attention. (**B**) Experiment 2: Functional MRI study. White blocks represent simple response time task, grey blocks choice response time task and black blocks are rest periods. tDCS = transcranial direct current stimulation.

before, during and after 20 min of 1 mA transcranial direct current stimulation (Fig. 1A).

Patients were seated 80 cm in front of a computer screen with their arms comfortably supported and performed four blocks of the response time task and four blocks of the grip force task during the baseline period, with an inter-block delay of ~30 s. Following baseline testing patients relaxed for 20 min during which tDCS electrodes were positioned on the scalp. tDCS then started and, after 20 s, patients recommenced behavioural testing. Four blocks of each task were performed during stimulation and two blocks after stimulation had ceased. Visual analogue scales were presented at the beginning and end of the session to assess subjective measures of attention, fatigue, discomfort and pain.

Motor tasks

For the response time task, subjects held a joystick comfortably positioned in their stroke-affected hand to respond to green circles appearing on a monitor until a response was made (40 cues per block; interstimulus interval = 1–3 s, randomly jittered at 500 ms intervals; Presentation software v14.5; Neurobehavioural Systems Inc.).

For the grip force task, subjects were instructed to grip a dynamometer (Noraxon Inc.) with their stroke-affected hand as strongly as possible in response to the cue 'Grip'. Cues were presented for 3 s after an initial 20 s rest (five cues per block; jittered interstimulus interval = 17–22 s during which the word 'Rest' was displayed).

Data analysis

For analysis of response times, any response times >2 s were excluded from further analysis. Subsequently, the mean and standard deviation for each block were calculated and any response times that deviated from the mean by more than ± 2 SD were excluded from further analysis. The mean and standard deviation for remaining response times from each block were then re-calculated.

For grip force, the maximum force recorded for each of the five responses was used to calculate mean grip force per block.

For both response times and grip force, average scores for the four prestimulation blocks were averaged to give a baseline measure. There was no difference between the four during-stimulation blocks and the two post-stimulation blocks [repeated measures ANOVA $F(1,12) = 1.19$, $P = 0.29$]. All blocks after the 20-min break were therefore averaged together to give a measure that we will refer to as 'post-stimulation' for simplicity, which includes blocks performed during and after stimulation.

The mean response time and grip force calculated for post-stimulation blocks were transformed into change ratios from the baseline in that session (e.g. $\%\Delta$response time = mean response time$_{stimulation}$/mean response time$_{baseline}$ $\times$ 100). Differences between sessions and over time were assessed using repeated measures ANOVA. Planned comparisons between each real stimulation condition and sham were performed using paired t-tests and were not corrected for multiple comparisons. All statistical analyses were performed using PASW Statistics v18.0 (IBM).

Experiment 2: Functional magnetic resonance imaging study

Patients participated in three functional MRI sessions on separate days. In all sessions subjects were scanned while performing a motor task before and after 10 min of 1 mA tDCS during which patients lay at rest (Fig. 1B). Two patients withdrew from the study before completion, one due to claustrophobia and one due to unrelated medical reasons.

Motor task

The visually cued motor task included a simple response time and a choice response time condition. Responses were made via a joystick held in the stroke-affected hand. The simple response time task required the patients to flex their wrists in response to any visual cue. The choice response time task, a simplified version of a task previously demonstrated to be dorsal premotor cortex-dependent in healthy controls (O'Shea *et al.*, 2007), required the patients to flex their wrist in response to a square and to extend it in response to a circle. A maximum extension of $10°$ was required. Task blocks were interleaved with rest blocks during which stimuli were displayed and the patient was asked to attend, but not respond, to the visual stimuli.

Eighteen blocks were presented in total in a fixed order. Each block consisted of six square and six circle stimuli displayed in a pseudo-random order (cue duration = 1500 ms; interstimulus interval = 500 ms or 2000 ms). The block type identifier was displayed for 1.5 s, followed by a blank screen for 1.5 s. Response times were analysed as described for response times in Experiment 1. Due to technical failure, behavioural data acquired during functional MRI were only available for seven patients for the anodal and cathodal tDCS sessions and eight for the sham tDCS session.

Magnetic resonance image acquisition

For all subjects except Patient 01, a 3T Siemens/Varian MRI system was used. Axial echo-planar volumes were acquired (43 mm $\times$ 3 mm axial slices, echo time = 28 ms, repetition time = 3000 ms, field of view = 192 $\times$ 192) before and after tDCS using a 1 channel receive head coil. No images were acquired during tDCS. A T_1-weighted anatomical image was also acquired for each subject (3D Turbo Flash, 165 mm $\times$ 1 mm axial slices, repetition time = 13 ms, echo time = 4.9 ms, inversion time = 200 ms, flip angle = $8°$, field of view = 256 $\times$ 256). For technical reasons Patient 01's scans were performed on a different 3T Siemens MRI system with a 1 channel head coil, using identical imaging parameters, but with 51 axial slices.

Magnetic resonance image analysis

Analysis was performed using tools from FMRIB Software Library (www.fmrib.ox.ac.uk/fsl) (Smith *et al.*, 2004). Images from the two patients with right hemisphere strokes were mirrored about the midline so that the lesioned hemisphere could be overlaid with images from the patients with left hemisphere strokes. All echo planar imaging data were de-noised using MELODIC prior to further analysis (Beckmann and Smith, 2004). Standard preprocessing and registration was applied (Supplementary Material).

For each subject, we acquired separate functional MRI runs for pre- and post-tDCS with anodal, cathodal and sham conditions. We analysed these data using three levels (see Supplementary Material for details): (i) within-session, within-subject time-series analysis; (ii) within-subject, between-session analysis to contrast effects of tDCS between stimulation conditions; and (iii) across-subject analysis of contrasts of interest.

Results

Experiment 1: Behavioural study

Experiment 1 was performed to study the effects of tDCS on response times and on grip force. There was no significant difference in prestimulation response times between the three stimulation conditions, $F(2,24) = 1.79$, $P = 0.189$. Comparing pre- and post-stimulation response times for the three stimulation conditions, we found a significant interaction between stimulation and time [repeated measures ANOVA $F(2,24) = 6.59$, $P = 0.005$] as predicted. We also found a significant main effect of stimulation [$F(2,24) = 4.18$, $P = 0.02$] but no main effect of time [$F(1,12) = 0.01$, $P = 0.9$]. As part of the analysis plan established prior to the experiment, we tested for changes relative to sham after each of the stimulation conditions. We found a significant response time decrease after anodal tDCS to ipsilesional M1 compared with sham stimulation [paired t-test, anodal versus sham $t(12) = 3.83$, $P = 0.002$] and, to a lesser degree, after cathodal stimulation to contralesional M1 [cathodal versus sham $t(12) = 2.20$, $P = 0.048$] (Fig. 2A).

In addition to comparing the change in response times due to tDCS with the sham stimulation condition, we were interested to know whether the response times were changed within session (i.e. post-stimulation compared with prestimulation). Anodal tDCS led to a significant decrease in response times [anodal pre- versus anodal post; $t(12) = 1.99$, $P = 0.04$]. There was no change in response times within session with either cathodal
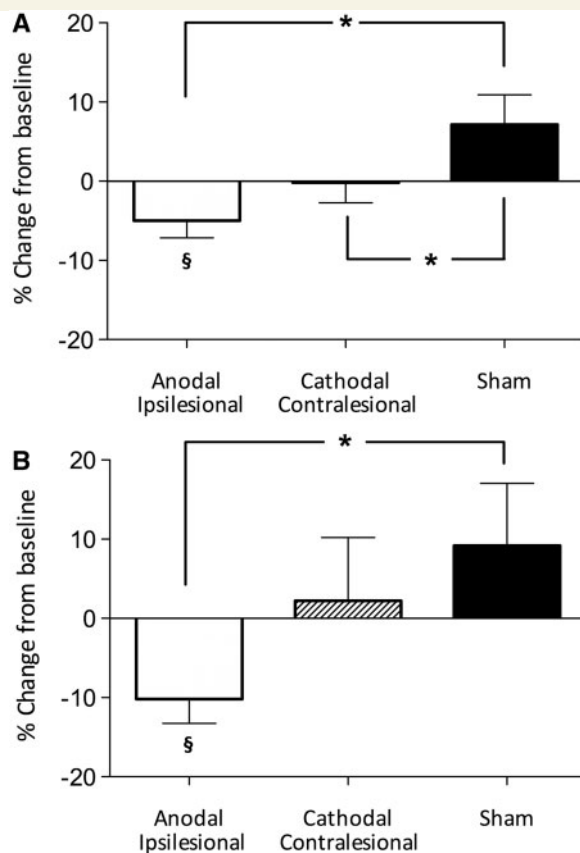
Figure 2 Behavioural effects of tDCS. (**A**) Experiment 1. Anodal stimulation to the ipsilesional M1 led to a significant decrease in response times. No significant difference was seen with cathodal stimulation. (**B**) Experiment 2. Anodal tDCS led to a significant shortening of response times. No change in response times was seen in response to cathodal tDCS. Columns represent mean ± SE. Significant differences ($P < 0.05$) between conditions (asterisk) and within session (section symbol) are highlighted.

tDCS [$t(12) = 0.11$, $P = 0.92$] or sham tDCS [$t(12) = 1.48$, $P = 0.16$].

Within session, response times did not change significantly over the post-stimulation blocks [repeated measures ANOVAs: anodal tDCS; $F(5,60) = 0.68$, $P = 0.63$; cathodal tDCS; $F(5,60) = 1.87$, $P = 0.1$; sham tDCS $F(5,60) = 0.60$, $P = 0.69$].

A significant correlation between baseline response time and percentage change in response time due to anodal tDCS also was observed; patients with slower baseline response times showed greater improvements in response time in response to stimulation ($r = -0.776$, $P < 0.01$).

There was no significant effect of stimulation on grip force (all $P > 0.1$). There also were no significant changes in attention, pain or discomfort scores over the course of the experiment as determined by the visual analogue scale scores [main effect of time $F(3,33)_{Attention} = 1.28$, $P > 0.29$; $F(3,33)_{Pain} = 1.34$, $P > 0.27$; $F(3,33)_{Discomfort} = 1.34$, $P > 0.27$]. Patients showed increased fatigue over time [$F(3,33) = 4.75$, $P = 0.01$], but this was not

affected by stimulation condition [time × stimulation interaction $F(6,66) = 1.52$, $P = 0.18$].

There was no significant effect of stimulation on the number of trials excluded from each block due to variability in response time: no main effect of stimulation [repeated measures ANOVA $F(2,24) = 0.291$, $P = 0.75$]; no main effect of time [$F(1,12) = 0.676$, $P = 0.453$]; and no interaction between stimulation and time [$F(2,24) = 1.39$, $P = 0.67$].

Experiment 2: Functional magnetic resonance imaging study

Experiment 2 was performed to define any cortical activation changes associated with the behavioural effects of tDCS on response times. Analysis of changes between pre- and post-stimulation trials in the simple response time task performed in the scanner demonstrated no significant difference in prestimulation response times between the three stimulation conditions $F(2,10) = 1.79$, $P = 0.216$. There was a significant interaction between stimulation and time [repeated measures ANOVA $F(2,10) = 6.38$, $P = 0.01$] as for Experiment 1. We found no significant main effect of stimulation [$F(2,10) = 0.2$, $P = 0.82$] and no main effect of time [$F(1,5) = 0.04$, $P = 0.83$]. Response times decreased following anodal tDCS to ipsilesional M1 compared to the sham condition [paired t-test, anodal versus sham $t(6) = 2.59$, $P = 0.04$], but no significant response time change after cathodal tDCS to contralesional M1 was found [paired t-test, cathodal versus sham $t(6) = 0.91$, $P > 0.3$] (Fig. 2B).

In addition to comparing the change in response times due to tDCS with the sham stimulation condition, we were interested to know whether the response times were changed within session (i.e. post-stimulation compared with prestimulation). Anodal tDCS led to a significant decrease in response times [anodal pre versus anodal post; $t(6) = 3.16$, $P = 0.01$]. There was no change in response times within session with either cathodal tDCS [$t(6) = 0.18$, $P = 0.85$] or sham tDCS [$t(7) = 1.35$, $P = 0.22$]. We also investigated the effect of stimulation on the number of responses removed from each session due to variability. There was no main effect of stimulation [repeated measures ANOVA $F(2,10) = 1.00$, $P = 0.4$], no main effect of time [$F(1,5) = 3.92$, $P = 0.1$] and no interaction between stimulation and time [$F(2,10) = 1.24$, $P = 0.33$].

There was no correlation between any clinical scores or lesion volume and behavioural improvement with either tDCS paradigm.

As expected, performance of the simple response time task compared to rest prior to stimulation was associated with bilateral activation of visuomotor areas (Supplementary Fig. 1). There were no significant differences between the baseline motor-related activation patterns in the three sessions. We first contrasted the motor-related activation patterns before and after each real tDCS condition compared with sham i.e. [(tDCS_Post–tDCS_Pre)–(sham_Post–sham_Pre)] using a voxel-wise analysis. After anodal tDCS applied to ipsilesional M1, task-related activity increased within the ipsilesional (stimulated) M1, bilateral dorsal premotor cortex and the supplementary motor area (Fig. 3A and Table 2). Cathodal tDCS applied to contralesional M1 was associated with a
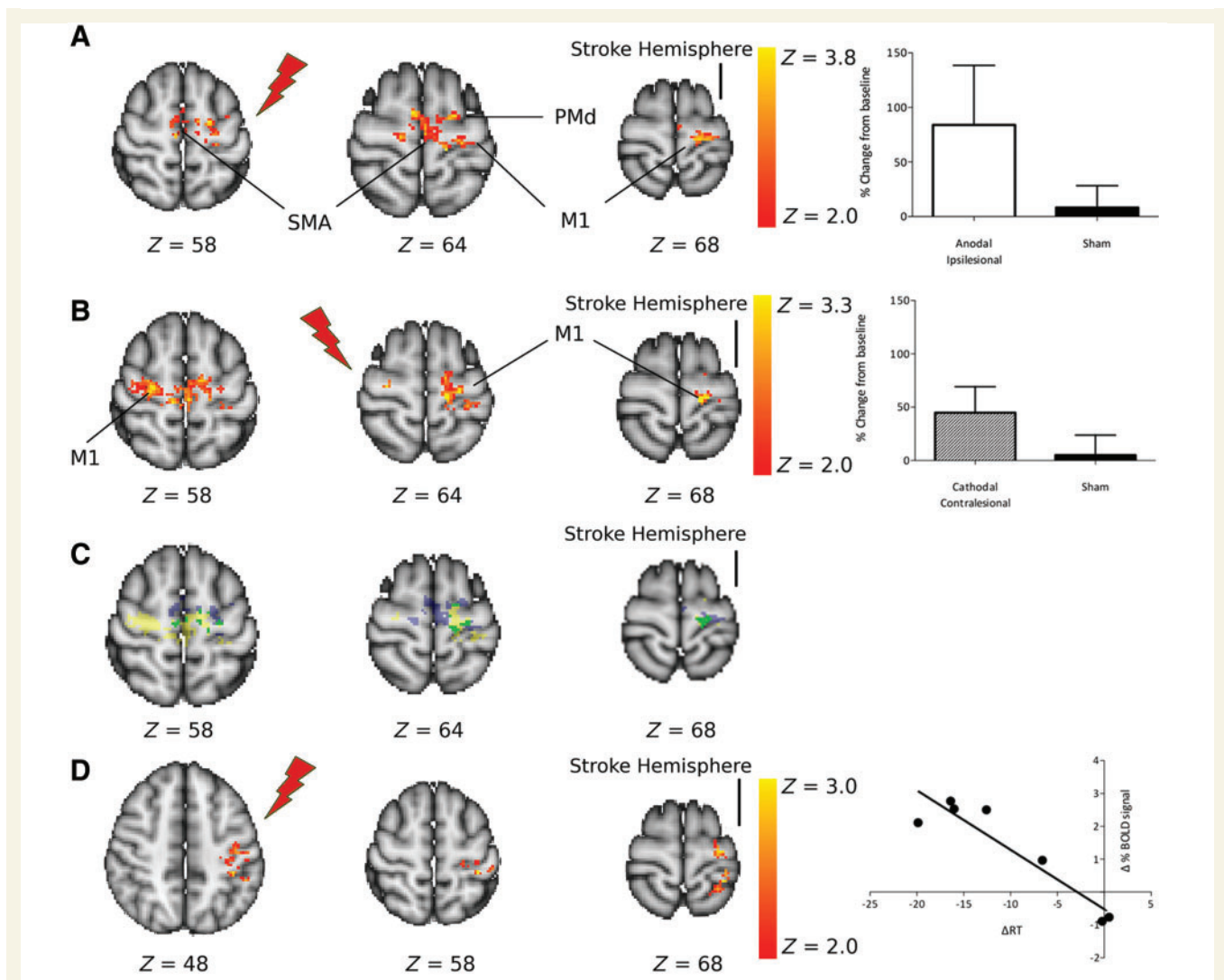
Figure 3 (**A**) Areas of increased motor-related activation in response to the simple response time task after anodal stimulation compared with sham [i.e. for the contrast (anodal post–anodal pre)–(sham post–sham pre)]. The column graph (*top right*) shows the mean change in activity within these suprathreshold regions and demonstrates a significant increase in activity within these areas after anodal stimulation and no change after sham stimulation. (**B**) Areas of increased motor-related activity in response to the simple response time task after cathodal stimulation compared with sham. The graph (*centre right*) demonstrates an increase in activity within these suprathreshold regions in response to cathodal stimulation but not to sham. (**C**) Areas of increased motor-related activity after anodal tDCS compared with sham (blue), increased motor-related activity after cathodal tDCS compared with sham (yellow) and areas of increased motor-related activity common to both stimulation conditions (green). (**D**) Areas of significant correlation between change in motor-related activation after anodal stimulation (i.e. the contrast anodal post–anodal pre) and change in response times after anodal stimulation. The plot (*bottom right*) demonstrates this relationship within in the areas shown. BOLD = blood oxygen level-dependent; PMd = dorsal premotor cortex; RT = response time; SMA = supplementary motor area.

significant increase in motor-related activity within the ipsilesional (unstimulated) M1, dorsal premotor cortex and supplementary motor area, as well as the contralesional (stimulated) M1 (Fig. 3B and Table 2). Common regions of increased motor-related activity in response to both anodal tDCS to ipsilesional M1 and cathodal tDCS to contralesional M1 were found in ipsilesional M1, dorsal premotor cortex and supplementary motor area (Fig. 3C and Table 2).

We then tested voxel-wise for a relationship between behavioural improvements on the simple response time task and cortical

activation increases in response to each real tDCS condition. For anodal tDCS to ipsilesional M1 we found a negative correlation; patients with larger decreases in response times showed greater increases in task-related cortical activation in the ipsilesional (stimulated) M1 (Fig. 3D and Table 3). By contrast, no significant correlation was found between response time change due to cathodal tDCS and voxel-wise functional MRI signal changes. To directly contrast the strength of this relationship between the two real stimulation conditions we compared the correlation coefficients from the voxel showing maximal correlation in each case.

Table 2 Regions of significantly increased activity in response to the simple motor task after tDCS when compared with sham

	Cluster size (mm³)	Maximum Z-score	MNI Coordinates of maximum Z-statistic		
			X	Y	Z
Increased functional MRI activity after anodal stimulation compared with sham					
Overall	4616	3.16	−18	−24	70
M1$_{Ipsi}$		3.16	−18	−24	70
PMd$_{Ipsi}$		3.05	−32	−2	60
SMA		2.75	−12	−16	60
Increased functional MRI activity after cathodal stimulation compared with sham					
Overall	6104	3.35	−16	−24	68
M1$_{Ipsi}$		3.35	−16	−24	68
M1$_{Cont}$		3.21	31	−22	56
S1$_{Ipsi}$		2.69	−18	−32	62
PMd		2.65	−20	−8	66
SMA		2.54	−4	−14	58
Volumes of overlap of increased functional MRI activity after anodal stimulation compared with sham and cathodal stimulation compared with sham					
M1$_{Ipsi}$	480				
SMA	224				
PMd	128				

Cont = contralesional; Ipsi = ipsilesional; MNI = montreal neurological institute; PMd = dorsal premotor cortex; SMA = supplementary motor area.

Table 3 Correlation between increased functional MRI activity after anodal stimulation and induced behavioural change

	Cluster size (mm³)	Maximum Z-score	MNI Coordinates of maximum Z-statistic		
			X	Y	Z
Overall	5400	3.01	−36	−36	46
M1$_{Ipsi}$		3.01	−36	−36	46
PMd$_{Ipsi}$		2.37	−50	−2	36

Ipsi = ipsilesional; MNI = montreal neurological institute; PMd = dorsal premotor cortex.

This demonstrated that the correlation between response time change and functional MRI signal due to tDCS was significantly stronger for anodal compared to cathodal tDCS (anodal tDCS maximum correlation $r = -0.934$, cathodal tDCS maximum correlation $r = 0.34$; Fisher's r-to-Z conversion $Z = -3.11$, $P < 0.001$). To ensure that this correlation between change in functional MRI signal due to anodal tDCS and change in response time was not driven by variations in baseline response time, we performed a partial correlation between the mean functional MRI signal change due to anodal tDCS within the suprathreshold region demonstrated in Fig. 3D and change in response time, correcting for baseline response time in the anodal tDCS session. The relationship between change in functional MRI signal and response time remained ($r = -0.902$).

We additionally assessed responses with a choice response time task. There was no change in choice response times and no change in choice response time task-related functional MRI activity in response to either tDCS condition.

Discussion

This study aimed to explore the cortical activation changes underlying behavioural improvement evoked by tDCS to the motor cortex in patients with stable, chronic disability after a first stroke. Both within and outside the MRI scanner we found that anodal tDCS applied to ipsilesional M1 improved response times across widely varying levels of recovery, confirming previous behavioural reports in more restricted patient groups (Fregni et al., 2005; Hummel et al., 2005, 2006).

For the first time, the immediate functional MRI brain activation changes associated with these behavioural improvements were characterized. Anodal tDCS to ipsilesional M1 was associated with increased task-related activity in the ipsilesional (stimulated) motor cortex, premotor cortex and supplementary motor area. Moreover, the degree of behavioural improvement immediately following tDCS was correlated with the stimulation-induced changes in functional MRI signal within the stimulated M1. It may be that the mechanisms underlying long-term behavioural improvements in patients are different from those demonstrated here. Repeated, multiple sessions of tDCS potentially lead to longer-lasting motor improvements (Boggio et al., 2007; Reis et al., 2009). One previous study using 5 days of motor training paired with a 'dual' stimulation montage found evidence for increased motor-related activity in ipsilesional M1 after the 5 day training period (Lindenberg et al., 2010). Future work should test whether this increased activity is also found for the conventional montage used here.

Both anodal tDCS applied to ipsilesional M1 and cathodal tDCS applied to contralesional M1 were associated with increased ipsilesional M1 activation and in our behavioural study both were associated with some degree of performance improvement, although for cathodal tDCS this improvement was only seen when contrasted with the sham tDCS session. When the regions of increased motor-related activity after anodal tDCS to ipsilesional M1 and cathodal tDCS to contralesional M1 were directly compared a region of overlap was demonstrated within the hand region of ipsilesional M1, highlighting this region as a possible anatomical substrate for the behavioural improvement seen in response to stimulation. However, univariate analyses of functional MRI data cannot easily provide insights into network hierarchies. Future studies using effective connectivity analysis (Marreiros et al., 2008) or using complementary modalities that provide greater temporal resolution, such as magnetoencephalography, could be used for this purpose.

It has been previously shown that anodal tDCS increases excitability within the stimulated region (Nitsche et al., 2000) and, in addition to glutamatergic effects, decreases the total γ-aminobutyric acid (GABA) pool within the stimulated region (Nitsche et al., 2005; Stagg et al., 2009a). As well as increases in glutamatergic signalling, decreases in GABA-ergic activity have been implicated

in behavioural improvements in rodent models of stroke (Clarkson et al., 2010). Future pharmacological studies could test whether GABA modulation is a critical mediator for the behavioural effects observed here, possibly explaining the smaller magnitude of the behavioural effects of cathodal tDCS applied to contralesional M1.

An earlier, smaller study previously had suggested possible behavioural improvements in patients following strokes after cathodal tDCS applied to contralesional M1 (Fregni et al., 2005). We partially replicated this observation in Experiment 1, but found that the behavioural effects of cathodal tDCS to contralesional M1 were much weaker than those of anodal tDCS to ipsilesional M1, as cathodal tDCS to contralesional M1 led to no absolute improvement in response times but did lead to a reduction of the increase in response times seen with sham tDCS, which we believe is a fatigue effect. Although motor-related brain activity during the simple response time task was increased in ipsilesional M1 with cathodal stimulation, in contrast to our findings for the anodal tDCS condition, no relationship between performance and functional MRI activity was found. The increased activity within the stimulated (contralesional) M1 in response to cathodal tDCS applied to contralesional M1, an inhibitory tDCS protocol, is in line with findings from our previous study in healthy controls (Stagg et al., 2009b), and, in inhibitory repetitive transcranial magnetic stimulation studies has previously been suggested to be the result of locally decreased synaptic efficiency (Lee et al., 2003).

It is not clear why there is a discrepancy between the magnitude of the effects of cathodal tDCS applied to contralesional M1 reported here and in the previous study, where cathodal tDCS applied to contralesional M1 was found to be as effective as anodal tDCS to ipsilesional M1 (Fregni et al., 2005). It is unlikely that the behavioural probes used in this study are insensitive to the effects of tDCS, as we and others (Hummel et al., 2006) have demonstrated robust significant effects on a simple response time task of anodal tDCS to ipsilesional M1. A potentially important difference between the current study and the previous work (Fregni et al., 2005), is that our patient group was more impaired. Increased activity in the contralesional hemisphere may be functionally important rather than maladaptive in more severely impaired patients (Johansen-Berg et al., 2002; Gerloff et al., 2006; Lotze et al., 2006).

We did not find any significant improvement in grip force following either stimulation condition. A previous study reported improved grip force (and response times) following anodal tDCS to ipsilesional M1 (Hummel et al., 2006). However, the patients in the current study were more severely impaired than those in previous reports. In our experience, moderately and severely impaired patients find maintaining the optimal posture for good task performance difficult. We did not find any effect of either stimulation condition on choice response time. It may be that the more moderately and severely impaired patients in this study found the wrist extension movement required for this task difficult. It is also possible that this dorsal premotor cortex-dependent choice response time task is not affected by tDCS to M1; even though the electrode position used might be expected to have some effects on at least the more caudal parts of dorsal premotor cortex. An alternative explanation for the effects of tDCS on response times seen is as reflecting a global change in attention, rather than a specific motor effect. We would consider this unlikely as no effect was seen on the choice-response time task and the number of responses excluded due to long reaction times in the simple response time task did not change with stimulation. However, we cannot rule out this possibility. Future studies testing alternative stimulation sites could explore in more detail the anatomical specificity of the effects found here.

Conclusion

Here we have provided the first demonstration that improvements in specific motor functions elicited by tDCS are associated with changes in motor cortical activity and shown that these include increased activity in the ipsilesional motor cortex. The functional relevance of the changes with anodal tDCS is suggested by the positive correlation between increases in ipsilesional M1 activity and improvements in performance on the simple response time task. By contrast to the behavioural improvements with anodal tDCS to ipsilesional M1, behavioural responses to cathodal tDCS to contralesional M1 were much smaller, and only seen when response time change was compared with sham tDCS. Future studies can extend this validation of functional MRI as a neurophysiological measure of response with tDCS to better understand task-dependent effects and to optimize potentially therapeutic stimulation paradigms.

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Supplementary material

Supplementary material is available at Brain online.

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