STROKE

# Ipsilesional anodal tDCS enhances the functional benefits of rehabilitation in patients after stroke

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**Anodal transcranial direct current stimulation (tDCS) can boost the effects of motor training and facilitate plasticity in the healthy human brain. Motor rehabilitation depends on learning and plasticity, and motor learning can occur after stroke. We tested whether brain stimulation using anodal tDCS added to motor training could improve rehabilitation outcomes in patients after stroke. We performed a randomized, controlled trial in 24 patients at least 6 months after a first unilateral stroke not directly involving the primary motor cortex. Patients received either anodal tDCS ($n$ = 11) or sham treatment ($n$ = 13) paired with daily motor training for 9 days. We observed improvements that persisted for at least 3 months post-intervention after anodal tDCS compared to sham treatment on the Action Research Arm Test (ARAT) and Wolf Motor Function Test (WMFT) but not on the Upper Extremity Fugl-Meyer (UEFM) score. Functional magnetic resonance imaging (MRI) showed increased activity during movement of the affected hand in the ipsilesional motor and premotor cortex in the anodal tDCS group compared to the sham treatment group. Structural MRI revealed intervention-related increases in gray matter volume in cortical areas, including ipsilesional motor and premotor cortex after anodal tDCS but not sham treatment. The addition of ipsilesional anodal tDCS to a 9-day motor training program improved long-term clinical outcomes relative to sham treatment in patients after stroke.**

## INTRODUCTION

Stroke is the leading cause of severe long-term disability. Spontaneous recovery typically plateaus within 3 to 6 months, but rehabilitation can be effective at improving motor outcome, even in the chronic phase. Motor rehabilitation approaches vary widely, but there is increasing support for programs that encourage active movement and use neuroscience principles of motor learning to facilitate progression (*1*). Although greater intensity and duration of training lead to greater recovery (*2*), delivery of one-to-one training is time-consuming and expensive. Thus, there is increasing interest in adjunct therapies to enhance responses (*3*).

Rehabilitation-mediated recovery depends largely on processes of learning and plasticity (*4*, *5*), so manipulations that promote plasticity might be expected to enhance rehabilitation outcomes. For example, anodal transcranial direct current stimulation (tDCS) to the motor cortex is known to enhance excitability (*6*), reduce local inhibition (*7*), and facilitate motor learning (*8*, *9*) in healthy individuals. We therefore predicted that application of anodal tDCS to ipsilesional motor cortex, when paired with motor training, would enhance rehabilitation outcomes after stroke by facilitating brain plasticity.

Although the effects of serial sessions using other tDCS configurations have been reported (*10*–*12*), there is limited evidence on the use of ipsilesional anodal tDCS. Two recent systematic reviews (*13*, *14*) provide tentative support for the use of ipsilesional anodal tDCS in chronic stroke, but studies have not used serial sessions of anodal tDCS concurrent with motor training. We therefore performed a double-blind randomized controlled trial of anodal tDCS as an adjunct to a daily motor training program, the Graded Repetitive Arm Supplementary Program (GRASP) (*15*), delivered over nine consecutive working days. In addition, serial multimodal magnetic resonance imaging (MRI) was undertaken to test whether an effective intervention was associated with increased activation of the ipsilesional hemisphere and associated structural changes. Long-term follow-up clinical and imaging assessments tested the persistence of observed changes in motor function.

## RESULTS

We conducted a stratified, double-blind, sham-controlled, parallel-group study designed to test the effects of anodal tDCS as an adjunct to motor training in chronic stroke patients (at least 1 month after single stroke affecting motor function). Of 1191 patients assessed for eligibility, 26 were randomized to receive either anodal tDCS or sham treatment, and 24 completed the intervention (fig. S1). Details of stroke patients who completed the intervention are given in Table 1, and the location of lesions is shown in fig. S2. Patient sex, age at intervention, time after stroke, lesion side, type, and volume did not differ between the anodal tDCS and sham groups (Table 1). No lesions included primary motor cortex (fig. S2).

Clinical assessments and MRI were carried out at multiple time points before and after the intervention period (fig. S3). Neuroimaging measures were also taken before and after the intervention. During the intervention period, participants conducted daily, supervised 1-hour sessions of GRASP (*15*) over 9 days. For the first 20 min of each session, tDCS electrodes were positioned on the participant's scalp to deliver either brain stimulation via tDCS or sham treatment.

### Improved clinical test scores in the anodal tDCS group compared to sham group

Clinical outcomes were assessed at multiple time points after the intervention (fig. S3) using three clinical measures: Upper Extremity

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**Table 1. Details of patients in the sham (top) and anodal tDCS (bottom) groups.** Group means (SD) or counts are given for each variable. No variable differed significantly between groups. M, male; F, female; L, left; R, right; C, cortical; S, subcortical. Muscle response Y/N refers to whether an observable muscle twitch from the affected hand could be evoked (Y) or not (N) after transcranial magnetic stimulation of the ipsilesional motor cortex.

| | Subject number | Sex | Age at intervention (years) | Time post-stroke (months) | Lesion location side/type | Lesion volume ($mm^3$) | Muscle response |
|---|---|---|---|---|---|---|---|
| Sham | 1 | F | 76 | 47 | L/S | 3,294 | Y |
| | 3 | M | 67 | 47 | R/S | 431 | Y |
| | 5 | M | 56 | 29 | R/C | 30,149 | Y |
| | 6 | M | 55 | 92 | L/S | 26,160 | Y |
| | 8 | M | 64 | 19 | R/C | — | N |
| | 11 | F | 79 | 68 | L/S | 14,822 | N |
| | 13 | M | 72 | 31 | R/C | 91,237 | Y |
| | 14 | M | 78 | 141 | R/C | 45,736 | Y |
| | 18 | M | 64 | 39 | R/S | 40,167 | Y |
| | 19 | M | 68 | 97 | R/S | 3,751 | Y |
| | 20 | M | 69 | 99 | R/S | 63,866 | Y |
| | 22 | F | 44 | 9 | R/S | 56,010 | N |
| | 23 | F | 77 | 18 | L/S | 10,548 | Y |
| | | | **66.8** | **56.6** | **4L/9R** | **32,181** | **3N/10Y** |
| | | | **(10.4)** | **(39.8)** | | **(28,232)** | |
| Anodal | 2 | M | 74 | 44 | R/C | 366,389 | Y |
| | 4 | M | 58 | 72 | L/S | 1,418 | Y |
| | 7 | F | 45 | 54 | R/C | 21,955 | Y |
| | 9 | M | 65 | 52 | R/S | 108,420 | Y |
| | 10 | M | 71 | 44 | R/S | 3,448 | Y |
| | 12 | M | 72 | 109 | R/S | 71,101 | Y |
| | 15 | M | 58 | 105 | R/S | 34,305 | Y |
| | 16 | F | 52 | 17 | R/S | 5,824 | N |
| | 17 | M | 70 | 41 | R/S | 735 | Y |
| | 21 | M | 53 | 6 | L/S | 11,061 | N |
| | 24 | F | 37 | 19 | L/S | 6,593 | N |
| | | | **59.5** | **51.2** | **3L/8R** | **57,386** | **3N/8Y** |
| | | | **(12.1)** | **(33.4)** | **2C/9S** | **(108,027)** | |

Fugl-Meyer Assessment (UEFM) (*16*), Action Research Arm Test (ARAT) (*17*), and Wolf Motor Function Test (WMFT) (*18*). Mean scores for each group and each time point on these clinical measures are shown in Table 2.

To test whether clinical scores increased in the anodal tDCS group compared to the sham group, each clinical score was subjected to multiple regression using the general linear model (GLM). The model variables were group (sham and anodal tDCS) and individual participant baseline scores for the respective clinical measure. By including baseline scores in the model, we ensured that variability in starting scores was taken into account. Plots of clinical scores for each group for each of the post-intervention time points are shown in Fig. 1.

To test for any effect of anodal tDCS on clinical outcomes, we first ran a single global test combining all three clinical measures and all four post-intervention time points and tested for a difference between the anodal tDCS and sham groups using Fisher's combined probability test, assessed with permutations. This showed greater scores in the anodal tDCS group than in the sham group [Fisher's $\chi^2(24) = 71.00$, $P = 0.008$]. Next, we ran the same test for each clinical measure separately, pooling across all post-intervention time points. We found higher scores for the anodal tDCS group compared to the sham group for both ARAT [$\chi^2(8) = 28.88$, $P = 0.031$, corrected for the three measures considered] and WMFT [$\chi^2(8) = 27.91$, corrected $P = 0.037$] but not for UEFM [$\chi^2(8) = 14.22$, corrected $P = 0.329$].

The time point of primary interest was the 3-month follow-up. At this time point, pooling across all clinical measures revealed greater scores in the anodal tDCS group compared to the sham group [$\chi^2(6) = 29.06$, $P = 0.004$, correcting for four time points]. At the 3-month follow-up, the mean absolute difference in the change in clinical scores from baseline between anodal tDCS and sham groups was 2.898 for

UEFM [95% confidence interval (CI), −2.136 to 7.932; $t(21) = 1.20$; $P = 0.550$], 5.763 for ARAT [95% CI, 1.560 to 9.966; $t(21) = 2.85$; $P = 0.045$], and 6.871 for WMFT [95% CI, 3.411 to 10.331; $t(21) = 4.13$; $P = 0.001$; these $P$ values were corrected for family-wise error rate across all tests, that is, four time points and three clinical scores]. At earlier time points and pooling across clinical measures using Fisher's test, we observed similar trends but these failed to reach significance after correction for the four time points [immediately post: $\chi^2(6) = 13.23$, corrected $P = 0.195$; 1 week: $\chi^2(6) = 12.05$, corrected $P = 0.248$; 1 month: $\chi^2(6) = 16.66$, corrected $P = 0.092$].

### Increased activity and gray matter volume in ipsilesional motor areas in the anodal tDCS compared to sham treatment group

MRI data were unavailable for patient 08 (due to claustrophobia) and patient 23 (due to scheduling constraints), both of whom were in the sham group. Neuroimaging analysis was therefore based on 11 participants per group. We considered functional MRI (fMRI) measures of activity during passive movement of the affected hand, voxel-based morphometry (VBM) measures of gray matter volume, and fractional anisotropy (FA) asymmetry measures of the corticospinal tract. For all voxel-wise analyses, images from patients with right hemispheric stroke were flipped about the midline after registration to standard space so that all lesions appeared on the left side of the image.

We first tested for correlations between clinical sores and imaging measures at baseline. No correlations were found for gray matter volume or movement-related fMRI activity. However, baseline asymmetry of corticospinal tract FA correlated negatively with all baseline clinical scores (Spearman test: ARAT $\rho = -0.77$, $P < 0.001$; UEFM $\rho = -0.79$, $P < 0.001$; WMFT $\rho = -0.78$, $P < 0.001$).

We next tested whether baseline imaging measures correlated with subsequent change in clinical scores due to the intervention. No significant relationships were found for fMRI or VBM measures. FA asymmetry correlated with subsequent behavioral improvements for UEFM ($\rho = 0.51$, $P = 0.015$) such that greater corticospinal tract asymmetry at baseline was associated with greater intervention-mediated behavioral improvements. However, this relationship did not survive covarying out the baseline UEFM score ($r = -0.67$, $P = 0.8$).

Comparing changes in imaging measures from before to after intervention between groups revealed greater increases in the anodal tDCS group compared to the sham group for fMRI activity and gray matter VBM but not for FA asymmetry. Specifically, larger increases in movement-related fMRI activation were found in the anodal tDCS group compared to the sham group (cluster $P < 0.05$, corrected), in regions including ipsilesional motor areas, both immediately after the intervention (Fig. 2A and table S1) and at 1-month follow-up (Fig. 2B and table S1). These fMRI results were similar after controlling for variation in gray matter (fig. S5). For gray matter VBM, greater increases were found immediately after the intervention in the anodal tDCS group relative to the sham group in ipsilesional premotor cortex, primary motor cortex, and the contralesional postcentral gyrus (Fig. 2C). Within this cluster, direction of mean change in gray matter volume was positive for the anodal tDCS group (0.0169 ± 0.021) and negative for the sham group (−0.0210 ± 0.023). However, when we tested across the whole brain for changes in gray matter volume from baseline in each group separately, we did not find any significant clusters. Therefore, although our results show a greater increase in gray matter in the anodal tDCS group compared to the sham group, there is no evidence regarding the direction of gray matter change in the absence of tDCS. No significant correlations were found between changes in clinical scores and changes in fMRI or VBM measures for any of the clusters. FA asymmetry values did not change after motor training [repeated-measures analysis of variance (RM-ANOVA), main effect of time, and group × time interaction: $P \geq 0.5$].

**Table 2. Mean scores for functional assessment measures for anodal tDCS and sham treatment groups.** In all assessments, higher scores indicate better performance. The UEFM ranges from 0 to 66, ARAT ranges from 0 to 57, and WMFT ranges from 0 to 75. Results are shown as means ± SD.

| | Anodal tDCS mean (SD) | Sham mean (SD) |
|---|---|---|
| **UEFM** | | |
| Baseline | 38.90 (15.89) | 36.42 (17.38) |
| Day 10 | 50.36 (11.16) | 45.53 (14.62) |
| 1 week | 48.91 (11.90) | 45.92 (15.52) |
| 1 month | 49.73 (12.67) | 46.46 (14.35) |
| 3 months | 48.18 (14.35) | 43.15 (16.29) |
| **ARAT** | | |
| Baseline | 20.27 (17.37) | 26.27 (20.17) |
| Day 10 | 29.91 (21.54) | 32.54 (21.54) |
| 1 week | 30.45 (20.67) | 33.08 (21.84) |
| 1 month | 30.27 (21.91) | 31.92 (20.64) |
| 3 months | 30.45 (20.92) | 31.31 (21.84) |
| **WMFT** | | |
| Baseline | 37.91 (20.21) | 39.65 (25.39) |
| Day 10 | 47.18 (17.46) | 48.00 (23.42) |
| 1 week | 49.45 (20.30) | 48.92 (24.44) |
| 1 month | 49.18 (19.08) | 46.54 (23.12) |
| 3 months | 48.36 (18.19) | 43.09 (23.78) |

## DISCUSSION

We report long-term improvements in upper limb ability in patients receiving repeated sessions of anodal tDCS to the ipsilesional motor cortex compared to the sham-treated group when tDCS was paired with motor training. We also found that these clinical improvements were associated with increased activation of ipsilesional motor cortical areas.

Previous proof-of-principle studies have shown that single sessions of anodal tDCS to the ipsilesional motor cortex temporarily improved motor function (*19*). For tDCS to have clinical relevance, however, it is critical that it be tested for long-term benefit. In healthy volunteers, repeated sessions of anodal tDCS paired with motor training provided long-lasting behavioral improvements (*20*). In chronic stroke patients, benefits have been found immediately or at 1 week after repeated sessions using other electrode configurations (*10*–*12*). One small study of subacute stroke patients found benefits of cathodal tDCS to contralesional motor cortex compared to sham treatment at 6 months of follow-up, but only a trend for beneficial long-term outcomes after

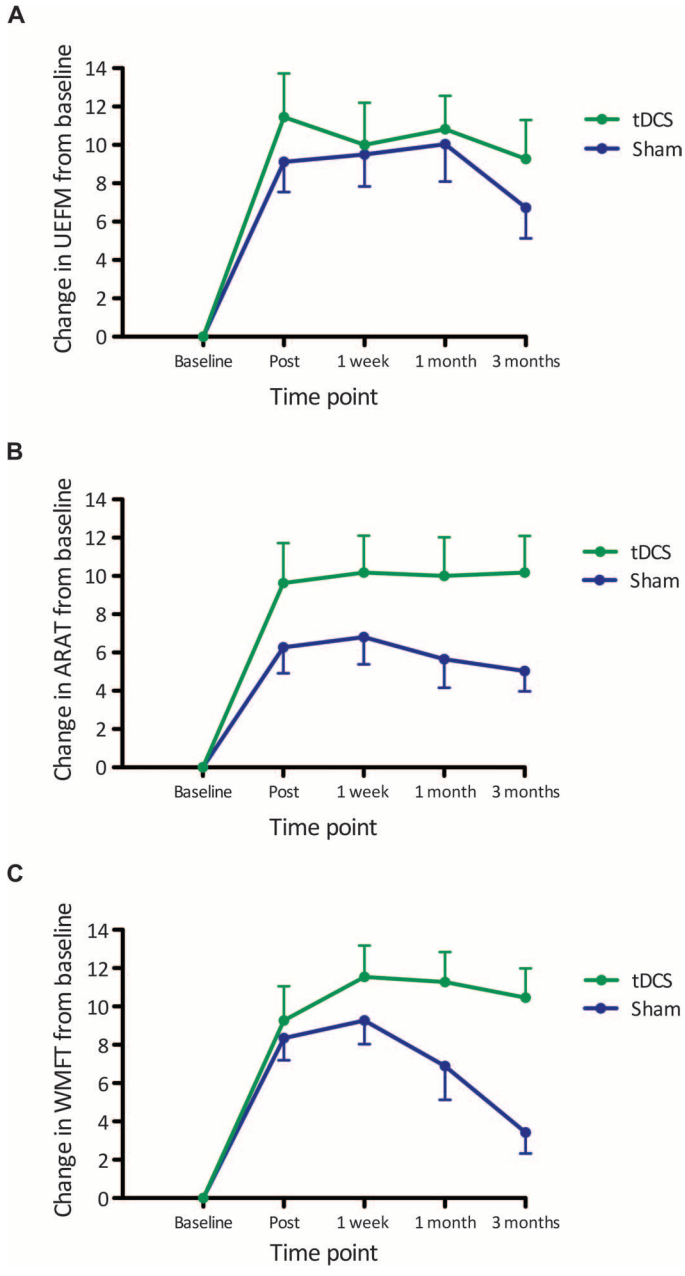


**Fig. 1. Increased clinical scores in the anodal tDCS group compared to the sham treatment group.** We assessed UEFM, ARAT, and WMFT clinical scale ratings before and at multiple time points after rehabilitation and either anodal tDCS or sham treatment. (**A** to **C**) Changes in scores from baseline for UEFM (A), ARAT (B), and WMFT (C) for anodal tDCS (green, $n = 11$) and sham treatment (blue, $n = 13$) groups, for the four post-intervention time points, after regressing out the respective baselines. Error bars represent SEM. Fisher's combined probability tests showed greater scores in the anodal tDCS group compared to the sham treatment group combined across all tests and all time points ($P = 0.008$). Considering each test separately, greater scores were found in the anodal tDCS group for both ARAT ($P = 0.031$) and WMFT ($P = 0.037$) but not for UEFM ($P = 0.329$). For the time point of primary interest, the 3-month follow-up, Fisher's combined probability test showed greater scores for the anodal tDCS group compared to the sham treatment group when combined across all tests ($P = 0.004$). Considering each test separately, greater scores were found in the anodal tDCS group at the 3-month follow-up for both ARAT ($P = 0.045$) and WMFT ($P = 0.001$), but not for UEFM ($P = 0.550$). All $P$ values were corrected for multiple comparisons across measures/time points as appropriate. Changes in clinical scores from baseline in the anodal tDCS and sham treatment groups are shown separately in fig. S4.

anodal tDCS (*21*). However, it is unclear whether cathodal tDCS to the contralesional hemisphere is suitable for all patients because some patients may depend on contralesional activation for movement of the affected hand (*22*, *23*). We therefore investigated the long-term effects of anodal tDCS on patients undergoing motor rehabilitation.

Clinical outcomes from a rehabilitation intervention can be manifested at different levels, including the domains of body function, activity, or participation. Focusing on a single domain may risk missing effects on other domains (*24*, *25*). To maximize our chances of detecting effects that could then be followed up in future targeted trials, we considered outcomes using three clinical scales in the domains of body function (UEFM) and activity (ARAT and WMFT). Whereas GRASP itself reduced impairment and improved activity (that is, UEFM, ARAT, and WMFT improved in both groups), tDCS only modulated activity improvements. Our findings therefore suggest that anodal tDCS to the ipsilesional hemisphere may exert its effects by enhancing activity and reducing functional limitations, rather than by changing the impairment.

Improvements in activity may be mediated not only by reductions in impairment but also through motor learning achieved through repetitive training on specific motor tasks. In line with others (*26*–*29*), our rationale for combining training strategies with adjunctive approaches depends on the fact that motor training programs such as GRASP involve motor learning and that motor learning is possible in stroke patients (*30*, *31*). Anodal tDCS applied to motor cortex has been shown to improve motor learning in healthy subjects (*20*) in part through local disinhibition (*7*).

Improvements in activity may be considered "compensation" as distinct from "true recovery," which can only occur if impairment is reduced and original pathways for movement are restored (*4*, *27*, *32*, *33*). This distinction is theoretically useful for understanding the level through which tDCS exerted its effects and is important for conceptualizing a theory-based rationale for how best to use tDCS in stroke rehabilitation. However, even compensation can be useful to patients if it allows them to perform movement tasks more effectively than before.

If anodal tDCS exerts its beneficial effects by enhancing learning, it is important to establish whether patients simply improve performance of specific trained movements or whether improvements can be generalized to nontrained items (*28*). There was some overlap between specific items included in the GRASP training and elements of the ARAT/WMFT assessment, and so, tDCS may in part have exerted its effects through boosting task-specific training. However, the specific movements trained in GRASP differ among patients depending on the level at which they enter the program, which was variable in this trial (the number of patients at level 1/level 2/level 3 was as follows: anodal tDCS, 5/2/4 patients; sham treatment, 4/3/6 patients). Further, there were items included in ARAT/WMFT that did not feature in GRASP. For example, adjusting a trained pinch grip to accommodate a different shaped object requires at least some degree of generalization. It will be important for future studies to design training and testing regimens

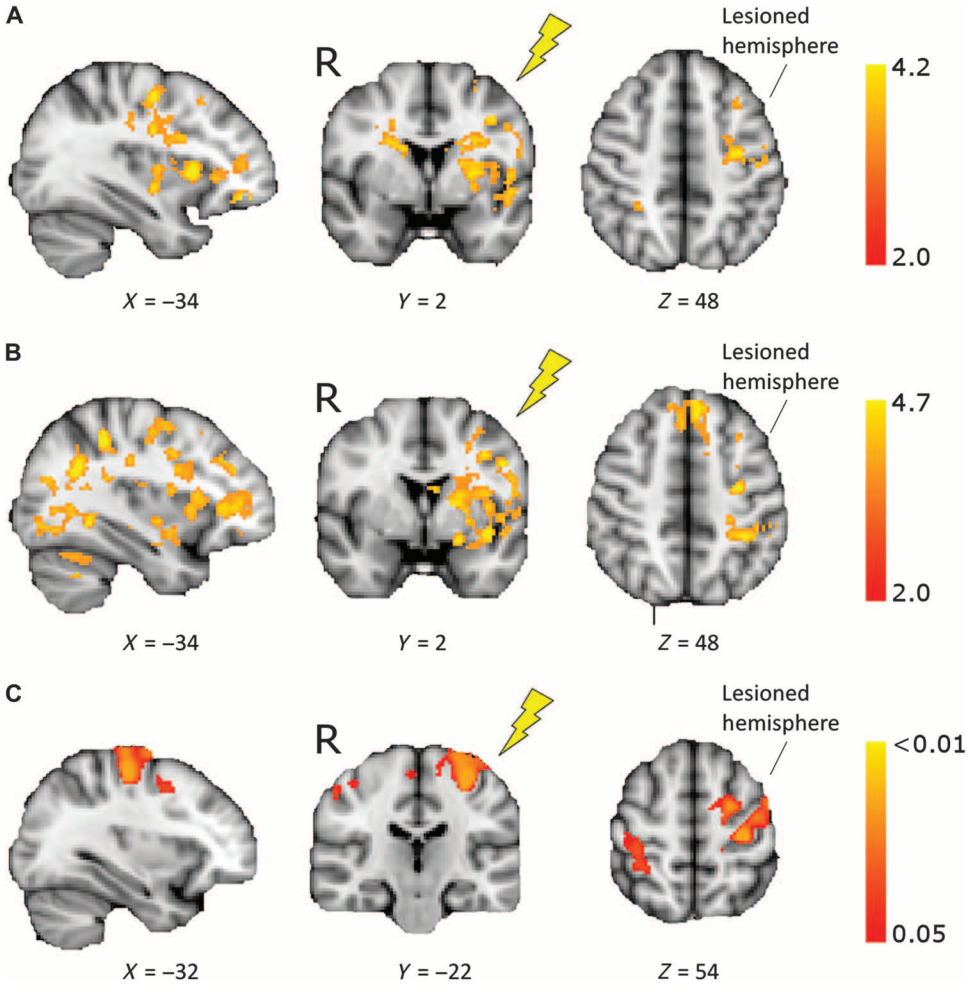


**Fig. 2. Increased fMRI activity and gray matter volume in the anodal tDCS group compared to the sham treatment group.** Shown are changes in fMRI activation and gray matter volume before and after intervention in stroke patients receiving motor rehabilitation plus anodal tDCS or sham treatment (after > before, anodal > sham, voxel-wise GLM; $P < 0.05$, corrected). Images are in radiological convention (R, right), and all lesions appear on the left of the image; yellow flash indicates the target for anodal tDCS. The *X*, *Y*, or *Z* coordinates for each brain slice are given below the slice. (**A**) Brain regions showing increases in fMRI activity during affected hand movement from baseline to immediately after intervention for the anodal tDCS group versus the sham treatment group. (**B**) Regions showing greater increases in movement-related fMRI activity for anodal tDCS versus sham group from baseline to 1-month follow-up. See table S1 for the location and *Z* statistic of peak voxels from fMRI analysis. (**C**) Brain regions showing increases in gray matter density as assessed by VBM, from baseline to immediately after intervention for the anodal tDCS group versus the sham treatment group.

to fully characterize the extent to which task-specific training versus generalization can be boosted by tDCS.

To test whether changes in brain structure and function could explain any observed clinical variation, we assessed neuroimaging measures. At baseline, worse clinical scores were associated with greater asymmetry of corticospinal tract microstructure, whereas no correlations were found between baseline clinical scores and fMRI or gray matter volume. We tested whether baseline neuroimaging measures predicted response to motor training, as shown previously (*34*). This is a clinically important question because such measures could potentially be used to target interventions at those patients who are most likely to benefit. Although we found correlations between baseline measures of corticospinal tract integrity and response to the intervention, these relationships did not increase predictive power above what could be explained by baseline clinical scores alone. Studies in larger and more variable patient groups will be required to assess the added value of such measures for clinical decision making.

We found greater increases in fMRI activation and gray matter volume in ipsilesional motor cortical areas in the anodal tDCS group compared to the sham group. Similar fMRI changes have been reported after a single session of anodal tDCS (*35*) and for serial sessions with other electrode configurations (*11*, *12*). A previous study reported an increase in gray matter with rehabilitative training alone (*36*), whereas here, although we found changes in gray matter between the two groups, within-group comparisons did not reveal clear evidence for a significant change over time for either group. We found no change in diffusion MRI measures of white matter microstructure with training between the groups, unlike a previous study showing new motor learning in healthy individuals (*37*).

Together with our previous observations of short-term training interventions (*38*), these findings suggest that the condition of residual brain pathways, as measured by diffusion MRI, may place some constraints on motor ability (as reflected by baseline correlations between FA asymmetry and clinical sores). Despite this, further gains in function may be possible and are more strongly associated with altered activation of motor cortical areas and gray matter structural changes, rather than with changes in white matter connectivity.

There are some limitations to this trial. First, although our sample size compares favorably to that of other studies of anodal tDCS in chronic stroke, a larger sample would provide greater power for identifying predictors of response. Second, the assessing researcher, who was blind to stimulation condition, also delivered the training. It is possible that knowledge of how participants had performed during the training session could have influenced their assessment of outcomes. Although we do not believe that this would unblind the assessor, it could potentially inflate any differences between participants that

began to be apparent during the training sessions. In addition, the treating assessor, who operated the tDCS equipment, was not blind to stimulation conditions. Although this experimenter played no role in the training and was not present at assessment sessions, it is possible that their behavior may have influenced the patient or the training researcher during training sessions. Future studies could make use of tDCS equipment that allows for stimulation protocols to be preprogrammed using a code to ensure that all those present are blind to stimulation conditions. Furthermore, because our hypothesis concerned the use of anodal tDCS as an adjunct to motor training, we included motor training in both arms of our study. Because we did not include an arm without motor training or tDCS, we cannot comment about the effect of the motor training itself, although this has been studied previously (*15*). Finally, because we did not test any healthy controls, our data do not provide evidence about whether the changes seen here would also be seen in healthy individuals.

Future larger studies are required to assess whether patient characteristics, such as baseline clinical scores or brain measures, could be used to stratify patients for maximal benefit. In addition, although our study was well tolerated, future trials using a less-intensive training regimen, perhaps consisting of two to three sessions per week, will be important to translate these findings into a clinical setting.

## MATERIALS AND METHODS

### Study design

This was a stratified, double-blind, sham-controlled, parallel-group study designed to test the effects of anodal tDCS as an adjunct to motor training in chronic stroke patients. Clinical tests were used to assess upper limb function before and after motor training paired with either real or sham tDCS. Neuroimaging measures were also taken before and after the intervention. The study is registered with ClinicalTrials.gov (NCT01414582) and was conducted in accordance with Consolidated Standards of Reporting Trials (CONSORT) guidelines. No replication was performed. The study was conducted in the UK, and all training and testing sessions took place within the John Radcliffe Hospital, Oxford. We recruited patients who were at least 6 months after a single unilateral ischemic or hemorrhagic stroke affecting motor function in the contralesional hand. Of 1191 patients assessed for eligibility, 26 were randomized to receive either anodal tDCS or sham treatment, and 24 completed the intervention (fig. S1 and Table 1).

All those who took part gave written informed consent to participate in accordance with local Research Ethics Committee approval after the nature and possible consequences of the study were explained. No participant had any history, signs, or symptoms of any other neurological condition, nor did they have dysphasia that limited communication. Exclusion criteria were as follows: previous stroke or stroke affecting the primary motor cortex, inability to provide informed consent due to severe language or cognitive impairment, and contraindications for tDCS. Participants with contraindications for MRI did not undergo MRI scans.

Clinical assessments and MRI were carried out at multiple time points before and after the intervention period (fig. S3). During the intervention period, participants conducted daily, supervised 1-hour sessions of GRASP (*15*) over 9 days. For the first 20 min of each session, tDCS electrodes were positioned on the participant's scalp to deliver anodal tDCS or sham treatment.

### Randomization and masking

After the first baseline assessment, the patients were randomized to the anodal or sham stimulation group. A random number generator was used to assign conditions in blocks of four, stratified by starting level on the motor training program (see below). Randomization was performed by a researcher (H.J.-B.) who was not involved in any baseline assessments, and allocation was communicated to one other researcher (U.A.). Motor training was carried out by a researcher blind to stimulation conditions (assessing researcher: C.A., with occasional cover by another blinded researcher, C.J.S.). All clinical assessments were scored by a researcher blind to stimulation conditions (C.A.). tDCS was delivered by a researcher who was aware of the stimulation conditions (treating researcher: U.A., with occasional cover by H.J.-B.) and who was not involved in any clinical assessments or motor training.

### Intervention: Motor training and anodal tDCS

Participants conducted daily, supervised 1-hour sessions of GRASP (*15*) over 9 days (Monday to Friday; Monday to Thursday). Patients began the program at one of three different starting levels, depending on the initial assessment of their motor abilities by a physiotherapist. There was a good spread of starting levels across patients, and our randomization procedure ensured that these were fairly distributed across both stimulation groups (number of patients within each group starting at level 1/level 2/level 3: anodal, 5/2/4; sham, 4/3/6). Progression was achieved by increasing repetitions and changing the weight or sizes of objects used. Motor training was supervised by the assessing researcher, who was blind to stimulation conditions. At the start of each session, this same researcher positioned two 5 × 7–cm electrodes, encased in saline-soaked sponges, on the participant's scalp, one centered over ipsilesional primary motor cortex (5 cm lateral to Cz: C3) and the other over the contralateral supraorbital ridge. The electrodes were connected to a DC stimulator (Eldith GmbH), which was controlled by the treating researcher. For anodal stimulation, the current was ramped up over 10 s, held at a constant 1 mA for 20 min, and then ramped down over 10 s. For sham stimulation, the current was ramped up over 10 s and then immediately switched off. Stimulation commenced at the same time as the motor training protocol. After 20 min, the electrodes were removed, and motor training continued for a further 40 min.

### Clinical assessments and statistical analysis

Clinical outcomes were assessed at multiple time points after the intervention (fig. S3) by a researcher blind to stimulation conditions (C.A.). The clinical outcome measures were the UEFM (*16*), ARAT (*17*), and WMFT (*18*). Statistical analysis of clinical scores was carried out using MATLAB R2013b. To test our primary hypothesis that greater clinical scores would be found in the anodal tDCS group, each score was subjected to a multiple regression using the GLM. The model variables were group (sham treatment or anodal tDCS) and the baseline for the respective score. We implemented nonparametric combination testing (*39*, *40*) to allow for combined tests that interrogate aggregate effects of anodal tDCS on scores and time points (see Supplementary Materials and Methods). We first ran a single global test, pooling the clinical scores and the post-intervention time points using the Fisher's combined probability test. To assess which clinical measures were showing any effect, we next repeated the procedure for each measure, pooling all post-intervention time points and computing family-wise error rate–corrected *P* values for each Fisher's $\chi^2$ combined statistic. Next, because we were

primarily interested in long-lasting clinical effects, we ran a similar nonparametric combination strategy using Fisher's test on data from the 3-month follow-up, pooling the three clinical measures and computing *P* values family-wise error rate–corrected across the four post-intervention time points. Finally, for each clinical measure, we reported the mean absolute difference and parametric CIs (95%) between anodal tDCS and sham groups at the 3-month follow-up and tested the significance of this difference.

To assess the time course and persistence of clinical gains within each group, we additionally ran paired *t* tests between baseline scores (for ARAT, UEFM, and WMFT) and scores at each post-intervention time point (immediate, 1 week, 1 month, and 3 months) as well as between the immediate post-intervention time point and all subsequent time points (1 week, 1 month, and 3 months). Permutation testing was run for each group separately (anodal tDCS and sham treatment) to generate *P* values, correcting for the three clinical scores and seven time point pairings assessed for each group.

### MRI acquisition and statistical analysis

MRI was performed on a 3T Verio scanner (Siemens) and included $T_1$-weighted MRI [magnetization-prepared rapid gradient echo: voxel size = 1 mm, isotropic; repetition time (TR) = 2040 ms; echo time (TE) = 4.7 ms; flip angle = 8°] and diffusion MRI (voxel size = 2 mm, isotropic; TR = 9600 ms; TE = 87 ms; two repeats of 60 directions, *b* value = 1000 s/mm$^2$; and eight volumes without diffusion weighting, *b* value = 0 s/mm$^2$). During fMRI (TR = 2410 ms; TE = 30 ms; flip angle = 90°; voxel size = 3 × 3 × 3 mm; 44 axial slices), passive flexion-extension of the stroke-affected hand was performed manually by a researcher, cued by a 1-Hz auditory cue. Movement and rest blocks alternated in 30-s periods.

Preprocessing and statistical analyses of MRI data were carried out using tools from the FMRIB (Functional Magnetic Resonance Imaging of the Brain) Software Library (FSL) (www.fmrib.ox.ac.uk/fsl) (see Supplementary Materials and Methods) (*41*). In brief, a standard FSL-VBM pipeline was used to preprocess data before analysis of longitudinal changes in gray matter. Images were then analyzed using permutation-based testing to test for statistical differences between time points and groups and for correlations with clinical scores. Threshold-free cluster enhancement was used to determine significance at $P < 0.05$, corrected for family-wise error. For clusters showing significant effects, mean voxel-wise estimates of gray matter were extracted for each subject for correlation with other variables.

Task fMRI data were preprocessed and analyzed using FEAT (fMRI Expert Analysis Tool) (see Supplementary Materials and Methods). A boxcar regressor modeling the 30-s task and rest blocks was used to create first-level statistical maps for each patient at each time point. Higher-level, mixed-effects analyses were then run using FLAME (FMRIB's Local Analysis of Mixed Effects) to compare activation maps across groups and time points and to test for correlations with clinical scores. *Z* statistical images were thresholded with an initial cluster-forming threshold of $Z = 2.0$ and a corrected cluster extent threshold of $P < 0.01$. For clusters showing significant effects, mean voxel-wise contrasts of parameter estimates were extracted for each subject for correlation with other variables.

Diffusion data were preprocessed using FMRIB's Diffusion Toolbox (FDT). For each patient and time point, mean voxel-wise values of FA were extracted from within standard-space corticospinal tract (CST) regions of interest. Asymmetry of the CSTs was calculated as follows: (contralesional CST FA − ipsilesional CST FA)/(contralesional CST FA + ipsilesional CST FA), with larger asymmetry reflecting relatively lower FA values in the ipsilesional CST. Statistical analysis of diffusion MRI measures of FA asymmetry in the CST was carried out using SPSS (Statistical Package for the Social Sciences, version 22). Measures were compared between groups and time points using RM-ANOVA, with $P < 0.05$ considered significant. Correlations between imaging variables and clinical scores were carried out using SPSS. Correlations were calculated using Pearson or Spearman correlation, depending on whether or not tests of normality were significant. A significance threshold of $P < 0.017$ (two-tailed) was used to correct for the three clinical scores considered.

## SUPPLEMENTARY MATERIALS

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Materials and Methods
Fig. S1. CONSORT flow diagram for study.
Fig. S2. Overlap in lesion volumes across patients for each group.
Fig. S3. Study timeline.
Fig. S4. Change in clinical scores compared to baseline for anodal tDCS and sham groups separately.
Fig. S5. No effect of correction for gray matter on fMRI results.
Table S1. Location and *Z* statistic of peak voxels from fMRI results.
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# Ipsilesional anodal tDCS enhances the functional benefits of rehabilitation in patients after stroke

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**Stimulating motor recovery in stroke**

Rehabilitation of movement after stroke requires repeated practice and involves learning and brain changes. In a new study, Allman *et al*. tested whether delivering brain stimulation during a 9-day course of hand and arm training improved movement in patients after stroke. The authors found greater improvements in movement in patients who received real compared to sham (placebo) brain stimulation. Better scores in patients who received real stimulation were still present 3 months after training ended. These findings suggest that brain stimulation could be added to rehabilitative training to improve outcomes in stroke patients.