Protocol Paper

Does connectivity of the stimulated motor network predict response to anodal transcranial direct current stimulation in people with stroke? A protocol for a double-blind randomised controlled trial

Authors: Ellana Welsby¹, Michael Ridding², Susan Hillier¹, Brenton Hordacre¹

Affiliations: 1. The Sansom Institute for Health Research, School of Health Sciences, The University of South Australia, Adelaide 5001, Australia.
2. The Robinson Research Institute, Adelaide Medical School, The University of Adelaide, Adelaide 5005, Australia.

Abbreviated title: Connectivity and tDCS response in stroke
Abstract

Background: Stroke can have devastating consequences for an individual's quality of life. Interventions capable of enhancing response to therapy would be highly valuable to the field of neurological rehabilitation. One approach is to use non-invasive brain stimulation techniques, such as transcranial direct current stimulation, to induce a neuroplastic response. When delivered in combination with rehabilitation exercises, there is some evidence that transcranial direct current stimulation is beneficial. However, responses to stimulation are highly variable. Therefore biomarkers predictive of response to stimulation would be valuable to help select appropriate people for this potentially beneficial treatment.

Objective: The objective of this study is to investigate connectivity of the stimulation target, the ipsilesional motor cortex, as a biomarker predictive of response to anodal transcranial direct current stimulation in people with stroke.

Methods: This study is a double blind, randomised controlled trial (RCT), with two parallel groups. A total of 60 participants with first ever ischemic stroke with motor impairment will undertake a two week (14 session) treatment for upper limb function (Graded Repetitive Arm Supplementary Program; GRASP). Participants will be randomised 2:1 to active:sham treatment groups. Those in the active treatment group will receive anodal transcranial direct current stimulation to the ipsilesional motor cortex at the start of each GRASP session. Those allocated to the sham treatment group will receive sham transcranial direct current stimulation. Behavioural assessments of upper limb function will be performed at baseline, post treatment, 1 month follow-up and 3 months follow-up. Neurophysiological assessments will include magnetic resonance imaging (MRI), electroencephalography (EEG) and transcranial magnetic stimulation (TMS) and will be performed at baseline, post treatment, 1 month follow-up (EEG and TMS only) and 3 months follow-up (EEG and TMS only).

Results: Participants will be recruited between March 2018 and December 2018, with experimental testing concluding in March 2019.

Conclusions: Identifying a biomarker predictive of response to transcranial direct current stimulation would greatly assist clinical utility of this novel treatment approach.
**Trial Registration:** Australia New Zealand Clinical Trials Registry; ACTRN12618000443291. Website [http://www.ANZCTR.org.au/ACTRN12618000443291.aspx](http://www.ANZCTR.org.au/ACTRN12618000443291.aspx)

**Keywords:** Stroke, Transcranial Direct Current Stimulation, Rehabilitation, Upper Limb, Magnetic Resonance Imaging, Electroencephalography
Introduction

Stroke is a global leading cause of death and disability. According to the World Health Organisation there were 6.7 million stroke related deaths in 2012, with 33 million stroke survivors living with persistent disability, and requiring long term care and secondary prevention measures [1]. A stroke affecting the sensorimotor network can lead to behavioural impairments, restricting capacity to perform various activities of daily living. As a result many stroke survivors require multi-disciplinary rehabilitation to help restore function [2]. Despite lengthy periods of rehabilitation, significant impairments often remain suggesting there is a need to do more to assist survivors of stroke.

Restitution of upper limb function following stroke is important to improve capacity to undertake activities of daily living and enhance quality of life. Underpinning functional restitution is a process known as neuroplasticity where both structure and function of the surviving brain tissue can change to optimise behaviour. Research indicates there may be a time limited window of enhanced neuroplasticity following stroke [3, 4]. This period of enhanced neuroplasticity following stroke has many similarities to those which occur during development where the brain is highly plastic and rapid learning occurs [3]. Delivering rehabilitative therapies during this period of enhanced neuroplasticity may provide opportunity for a more complete recovery. It is generally considered that this period of enhanced neuroplasticity occurs in the acute or sub-acute post stroke period [4]. In support of this, behavioural evidence indicates that therapy delivered early after stroke is more effective than that delivered in the chronic post stroke period [3, 5, 6].

One interesting approach to stroke rehabilitation is to attempt to re-establish a period of enhanced neuroplasticity to boost effects of therapy in people with stroke. Non-invasive brain stimulation techniques, such as transcranial direct current stimulation (tDCS), are a novel approach which may be able to facilitate neuroplasticity in the brain. It is thought that tDCS is capable of altering the level of intrinsic postsynaptic activity depending on the direction of current flow [7, 8]. When applied to the primary motor cortex (M1), anodal tDCS increases cortical network
excitability and cathodal tDCS decreases cortical network excitability. Changes in excitability induced by tDCS are thought to be mediated by long term potentiation and long term depression-like synaptic plasticity [7, 8]. Several studies have demonstrated functional improvements in stroke patients following plasticity protocols applied to the lesioned motor cortex [9, 10]. However, recent reviews highlight that at the group level tDCS does not provide additional benefit to therapy [11]. Upon further investigation, it appears responses can be highly variable between individuals, suggesting this is not a one-size-fits-all treatment. Several factors are known to influence the response to tDCS including properties of the stimulated brain network, genetics and endogenous cortisol levels [12, 13]. Recently we demonstrated that connectivity of the stimulated network was a strong predictor of response to anodal tDCS in healthy adults [14]. Using electroencephalography (EEG) we found that connectivity between electrodes overlying the stimulated primary motor cortex and the ipsilateral parietal cortex in the high beta frequency (20-30Hz) predicted 69% of variability in the neuroplastic response to anodal tDCS using a leave-one-out and predict analysis. Along similar lines, connectivity of the stimulated ipsilesional motor network in alpha frequency (8-13Hz) is strongly associated with the change in corticospinal excitability following a tDCS in people with stroke [15]. It may be that connectivity of the network targeted by tDCS can be a useful predictor of response to brain stimulation therapy. Indeed, this may be even more critical following stroke, where damage as a result of the lesion can interrupt functional connectivity [16].

The primary objective of this study is to determine whether connectivity of the cortical target for tDCS modulates responses to this intervention in people with stroke. The secondary objectives of this study are to: 1) Determine whether facilitatory tDCS applied to the ipsilesional hemisphere in combination with an upper limb exercise program provides greater behavioural improvement compared to sham stimulation; and 2) Determine whether additional neurophysiological characteristics such as lesion size, cortical excitability and white matter integrity modulate response to tDCS. We hypothesise that response to anodal tDCS will be variable: participants who have greater functional connectivity of the ipsilesional motor network will have stronger responses to the stimulation as shown by a greater increase in upper limb
function following the intervention period. Outcomes from this study will have important implications for clinical translation of tDCS in stroke rehabilitation. The ability to select people who will respond to this therapy could substantially improve clinical translation of this treatment approach which may have a profound impact upon stroke rehabilitation.

Methods

The SPIRIT (Standard protocol Items: Recommendations for interventional trials) recommendations were referenced when developing this protocol. This protocol has been registered at the Australian and New Zealand Clinical Trials Registry (ACTRN12618000443291).

Study Design

This study is a double blind, randomised controlled trial (RCT), with two parallel groups. Both outcome assessors and participants will be blind to allocation. Randomisation will be performed by using a computerised sequence generation by an external researcher. As our primary research aim is to investigate brain connectivity of participants allocated to the active treatment group, allocation will be weighted 2:1 towards the active treatment group. A sham treatment group will be used as a comparator to demonstrate effectiveness of this intervention at the group level and to demonstrate that brain connectivity is not associated with response to sham tDCS.

The study protocol has been approved by the University of South Australia Human Research Ethics Committee (application identification 0000036781; approved 19th May 2017). Recruited participants will provide written informed consent in accordance with the World Medical Association Declaration of Helsinki.

Participants and recruitment

Stroke participants will be recruited from the community and a database of willing volunteers. Inclusion and exclusion criteria are stated in table 1.
Table 1: Study inclusion and exclusion criteria.

<table>
<thead>
<tr>
<th>Inclusion Criteria</th>
<th>Exclusion Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Aged ≥ 18 years of age</td>
<td>• TMS and tDCS safety exclusion criteria as per international guidelines</td>
</tr>
<tr>
<td>• At least 3 months post first ischemic stroke with motor impairment</td>
<td>• MRI safety exclusion criteria</td>
</tr>
<tr>
<td>• Mild to moderate impairment of the upper limb</td>
<td>• Neglect, apraxia or shoulder pain (&gt;4 out of 10 on pain VAS) that would affect</td>
</tr>
<tr>
<td>• Supportive family, friends or carers willing to actively assist and motivate</td>
<td>the ability to undertake a 1-hour upper limb exercise program.</td>
</tr>
<tr>
<td>across the two-week intervention</td>
<td>• Language and cognitive impairment that would limit ability to communicate with the</td>
</tr>
<tr>
<td>• Active wrist extension of at least 5 degrees</td>
<td>research team via video conference</td>
</tr>
<tr>
<td>• Active index finger flexion of at least 10 degrees</td>
<td>• Participation in a concurrent research study or clinical program for upper limb</td>
</tr>
<tr>
<td>• Modified Ashworth scores of &lt; 4</td>
<td>rehabilitation.</td>
</tr>
</tbody>
</table>

TMS, transcranial magnetic stimulation; tDCS, transcranial direct current stimulation; MRI, magnetic resonance imaging; VAS, Visual analogue scale.

**Sample size**

Our primary aim is to determine characteristics of the sensorimotor network at baseline that may predict response to anodal tDCS in people with stroke. Therefore, our sample size calculation was based on pilot data of 10 people with stroke where we observed a medium to large effect size for a correlation between baseline high beta frequency connectivity and change in cortical excitability following anodal tDCS [15]. Using this effect size with alpha = 0.05 and power of 95% we determined a sample of n = 31 would be required in the Active treatment group. However, given the nature of this home-based treatment and longer follow-up study period, we are
allowing for a 30% drop-out rate and will aim to recruit $n = 40$ into the Active treatment group.

**Experimental Protocol**

Participants will attend 6 experimental sessions as outlined in Figure 1. Session one will be conducted at the Clinical Research and Imaging Centre (CRIC; Dr Jones and Partners, South Australian Health and Medical Research Institute) where magnetic resonance imaging (MRI) sequences will be performed to obtain structural, diffusion and functional images.

Session two will be conducted within five days of the initial MRI scan at the University of South Australia, City East Campus, Clinical Trials Facility. Participants will be encouraged to attend this experimental session with a supportive family member, friend or carer. Participants will undergo baseline neurophysiological and behavioural outcome assessments and be provided with a home tDCS kit (NeuroConn DC-Stimulator Mobile, NeuroConn GmbH, Ilmenau, Germany). The participant and their support person will be trained in the use of the home tDCS, iPad and the Graded Repetitive Arm Supplementary Program (GRASP) exercises. Information sheets will be provided for use of both the home tDCS equipment and iPad. This information will detail correct use of equipment, including locating the correct spot for tDCS electrode position which will be marked on the scalp with permanent marker. To facilitate training, the first of 14 treatments will be undertaken at the Clinical Trials Facility under the supervision of a research staff member.

Experimental sessions three, five and six are respectively performed immediately, one month and three months following the final home tDCS treatment. At these sessions, participants will undergo neurophysiological and behavioural outcome assessments. Experimental session four is a follow-up MRI session and will occur within five days of the final tDCS treatment.
Figure 1: Schematic diagram of experimental sessions. Sessions listed above the timeline are to be conducted at the University of South Australia, City East Campus, Clinical Trials Facility. Those listed below the timeline are to be conducted at the Clinical Research and Imaging Centre, South Australian Health and Medical Research Institute.

tDCS, transcranial direct current stimulation; EEG, electroencephalography; TMS, transcranial magnetic stimulation; ARAT, action research arm test; FM, Fugl-Meyer; FLAIR, fluid-attenuated inversion recovery; MRI, magnetic resonance imaging.

**Intervention**

**Graded Repetitive Arm Supplementary Program**

All participants will be provided with a home exercise program using the GRASP. The GRASP level (grade 1-3) will be individualised by a qualified Occupational Therapist based on impairment of the upper limb. GRASP will be performed for 1 hour daily over a two-week period (14 sessions).

**Transcranial direct current stimulation**

Participants will also be provided with a home tDCS kit which will be pre-programmed for 14 sessions of active or sham stimulation using a study code to facilitate research staff blinding. Participants will be unable to modify or observe any
settings of the home tDCS devices. To initiate stimulation, participants simply position electrodes on the head and press the start button. Those randomised to the 'Active' arm of the study will receive tDCS while simultaneously performing the GRASP exercises. TDCS will be delivered for 20 minutes at the start of the 1-hour GRASP program. TDCS involves weak direct current passing between two surface electrodes placed on the scalp. In this study, the electrodes will be positioned with the anode over the ipsilesional M1 and cathode over the contralateral supraorbital region. TDCS will be applied at intensity of 1mA for 20 minutes daily for two weeks (total of 14 sessions) at home. Stimulation will be ramped up from 0mA to 1mA over the first 30 seconds and down from 1mA to 0mA over the final 30 seconds.

Participants randomised to the 'Sham' arm of the study will receive Sham tDCS while undertaking the 1-hour individualised GRASP exercises. Electrodes will be positioned in the same location as the active tDCS group. Sham tDCS mimics the sensation of stimulation without changing cortical excitability. Sham tDCS will ramp current up from 0mA to 1mA over the first 30 seconds before ceasing for the following 19 minutes. The final 30 seconds will ramp from 1mA down to 0mA. This approach has been shown to provide an effective sham stimulation protocol [17].

**Compliance monitoring**

Several strategies will be employed to monitor protocol compliance and ensure correct use of home tDCS (see Box 1). The number of completed tDCS sessions and daily duration of GRASP will be reported.

---

Box 1: Strategies to facilitate protocol compliance

- **Support person** – A support person will attend training for home tDCS and GRASP exercises to assist and motivate the participant as required across the intervention.
- **Initial treatment under supervision** – The first treatment will be completed under supervision to ensure correct use of tDCS.
- **tDCS electrode position marked on scalp** – The correct positioning of tDCS electrodes will be marked on the scalp with a permanent marker to facilitate home application.
- **Information sheet** - Step-by-step instructions for use of the home stimulator.
- **Video conference** – In real time, confirm correct tDCS usage, provide motivation and progress individual exercise programs or GRASP grade.
- **Exercise diary** – Record daily completion of tDCS and GRASP. Includes recording duration of GRASP therapy, motivation, fatigue, perceived exercise difficulty.

### Adverse events and assessment of blinding
At the completion of the two week tDCS intervention, participants will be asked to complete a questionnaire to identify any adverse events and establish whether participant blinding was successful. In accordance with current recommendations [18], we will ask participants to rate on a scale of one to four (1 = absent, 2 = mild, 3 = moderate, 4 = severe) the presence of the following symptoms; headache, neck pain, scalp pain, tingling, itching, burning sensations, skin redness, sleepiness, trouble concentrating, acute mood change, other symptoms. We will also ask participants to determine if those symptoms were present, to what extent do they believe it to be related to using tDCS (1 = none, 2 = remote, 3 = possible, 4 = probable, 5 = definite). To determine effectiveness of blinding we will ask participants to indicate if they believe they received active stimulation (yes/no).

### Outcome Measures
Participant demographics and clinical characteristics including age, gender and time since stroke will be recorded and compared between active and sham groups.

### Assessments of upper limb function
The primary outcome measure for this study is a change in upper limb impairment as measured with the Fugl Meyer-Upper Extremity (FM-UE) assessment. The FM-UE is a commonly used, validated and reliable measure of sensorimotor impairment [19].
The FM-UE is considered as one of the most comprehensive quantitative measures of motor impairment following stroke.

Secondary outcome measures will include the Action Research Arm Test (ARAT) and grip strength. The ARAT is a valid and reliable measure of hemiplegic upper limb function [20]. It provides a quantitative measure of upper limb function for domains of grip, grasp, pinch and gross arm movement. Grip strength is associated with motor cortical output and motor recovery [21]. We will measure grip strength using a hand dynamometer (SH5001 Saehan Hydraulic Hand Dynamometer, Saehan Corp., Korea). The best (maximal) grip of three attempts will be recorded.

Outcome assessors will be blind to group allocation and have completed online training for FM-UE and ARAT assessments through the University of California Irvine. Training outcome assessors with this approach has been shown to improve accuracy and reduce variance of the FM-UE and ARAT [22, 23].

**Neurophysiological Testing**

**Electroencephalography**

Functional connectivity between brain regions will be assessed with high density electroencephalography (EEG). We will record EEG at rest using a 64-electrode cap. Impedance will be kept below 5kΩ while recording. Artefact rejection will be performed prior to analysis using independent component analysis. Non-physiological artefacts will be identified using an automated and objective method to remove assessor bias [24]. Functional connectivity between electrodes will be determined using the debiased weighted phase lag index (dwPLI), which is a conservative estimate of connectivity based on phase consistency and biasing against zero phase lag relationships, limiting detection of spurious measures of connectivity [25]. Frequency bands of interest are the Alpha band (8-15Hz) and Beta band (16-31Hz) as they are associated with sensorimotor function [14, 26, 27]. For a given frequency, a dwPLI value of 1 indicates maximal phase coupling, while a value of 0 indicates no phase coupling. Connectivity analyses will be performed in MATLAB 9.2.0 (MathWorks, Inc., Natick, MA) using both EEGLAB [28] and FieldTrip toolboxes [29].
**Transcranial magnetic stimulation**

Single pulse transcranial magnetic stimulation (TMS) will be used to quantify corticomotor excitability of the ipsilesional primary motor cortex. Monophasic (posterior to anterior current flow) TMS pulses will be delivered with a Magstim 200 stimulator (Magstim, Whitland, UK) via a figure-of-eight coil (90 mm external wind diameter). The coil will be placed tangentially over the scalp with the handle pointing 45° posterolateral. Surface electromyography (EMG) will be used to record motor evoked potentials (MEPs) from the first dorsal interosseous muscle of the paretic hand with electrodes positioned in a belly-tendon montage. Suprathreshold stimuli will be delivered over the ipsilesional hemisphere to identify the optimal position for evoking a MEP from the first dorsal interosseous muscle of the paretic hand. For participants where MEPs cannot be evoked even at maximal stimulator output as a result of the stroke, we will document that a measure of corticospinal excitability was not obtainable at that experimental session. For participants where MEPs could be evoked, the optimal site will be marked on the scalp using a felt-tip marker to ensure consistent coil placement during data collection. Resting motor threshold (RMT) will then be determined and is defined as the minimum stimulus intensity required to evoke a MEP of at least 50μV in at least 5 of 10 consecutive trials. Thirty stimuli, not contaminated by pre-stimulus EMG, will then be obtained at 120% RMT (inter-stimulus interval 4.5-5.5s) with the average, peak-to-peak amplitude, determined as a reliable measure of corticospinal excitability [30].

**Magnetic Resonance Imaging**

MRI will be performed at the Clinical and Research Imaging Centre (CRIC) located at the South Australian Health and Medical Research Institute with a Siemens 3T MAGNETOM Skyra scanner (Siemens, Erlangen, Germany). Standard MRI safety screening will be performed to ensure included participants are safe for MRI. At the pre-intervention MRI session, the imaging protocol will have a duration of 45 minutes and include T1 MPRAGE and T2 FLAIR weighted images, diffusion tensor imaging, resting state functional MRI (rs-fMRI) and task fMRI. At the post-intervention MRI
session, the imaging protocol will have a duration of 30 minutes and include T1 weighted images, resting state fMRI and task fMRI.

The imaging protocols are as follows; T1 weighted images (MPRAGE, voxel 1mm x 1mm x 1mm, TR = 2300ms, TE = 2.98ms, flip angle = 9°), T2 weighted FLAIR images (voxel 1mm x 1mm x 1mm, TR = 5000ms, TE = 393ms), diffusion MRI (voxel 2mm x 2mm x 2mm, TR = 4200ms, b-value = 0 and 2000 s/mm²), rs-fMRI (voxel 2.4mm x 2.4mm x 2.4mm, TR = 735ms, TE = 36ms, 2 repeats of 6 minutes duration, 490 measurements for each), and task fMRI (voxel 2mm x 2mm x 2.5mm, TR = 3000ms, TE = 30ms, 4.44 minutes duration). During task fMRI participants will be presented with a visual cue to squeeze a stress ball in their paretic or non-paretic hand, with blocks alternating every 30 seconds and repeated four times per hand. Pre-processing and statistical analyses of MRI data was carried out using tools from the FMRIB Software Library (FSL) [31].

**Statistical analysis plan**

Normality of data will be confirmed using Shapiro-Wilk normality tests. Where required, data will be normalised using transformations or non-parametric statistics applied. Participant demographics and clinical characteristics will be compared between active and sham groups. The effect of the intervention on behavioural and neurophysiological outcome measures will be investigated with a 2 Group (Active, Sham) x 4 Time Point (Baseline, Post Intervention, 1 Month Follow-up, 3 Month Follow-up) repeated measures ANOVA. Regression modelling will be used to identify biomarkers associated with response to anodal tDCS. A model with independent variable of functional connectivity will be compared to model(s) combining functional connectivity with additional neurophysiological characteristics to provide improved fit to account for variance in anodal tDCS response using the Bayesian information criteria [32]. Where appropriate, predictive capacity of the generated model will be investigated using a leave-one-out cross validation. This cross validation will be performed on participants allocated to both active and sham treatment groups to demonstrate that the predictive model is specific to stimulation
Statistical testing will be performed using SPSS (IBM corp. Version 24.0) and significance level will be $p \leq 0.05$.

**Results**
As of April 2018, eleven participants have been enrolled into the study with five beginning experimental testing. It is anticipated that the final participant enrolment will occur in December 2018, with data collection completed in March 2019. At the conclusion of the study, results will be disseminated through publication in scientific journals and presentations and conferences.

**Discussion**
Adjuvant therapies, such as tDCS, are critical to improving potential for motor function recovery following stroke. To date the response to tDCS has proved highly variable and this has limited clinical translation. This is likely due, at least in part, to stimulation being applied without consideration of individual motor network characteristics. This study will be a significant step forward in the development of precision approaches for use of brain stimulation in stroke rehabilitation. This will be achieved by providing evidence for biomarkers of brain connectivity to selectively apply tDCS to those stroke patients who will benefit most. Future work could lead to individualised brain stimulation protocols based on motor network connectivity and clinical presentation. This body of work has potential to enhance functional outcomes for a population who present a significant social and economic burden and are desperate for improved rehabilitation services.

**Acknowledgements**
This was supported by the Sylvia and Charles Viertel Charitable Foundation Clinical Investigator Award (VTL2016CI009). BH is funded by and National Health and Medical Research Council (NHMRC) fellowship (1125054). EW led the writing of this manuscript. BH conceived the study design and was successful in obtaining funding to support this research. MR and SH contributed to study design and reviewed manuscript. All authors approved the final manuscript.
Conflicts of Interest
None declared.

Abbreviations
ARAT: action research arm test
CRIC: clinical research and imaging centre
dwPLI: debiased weighted phase lag index
EEG: electroencephalography
FLAIR: fluid-attenuated inversion recovery
FM-UE: fugl meyer-upper extremity
GRASP: graded repetitive arm supplementary program
MRI: magnetic resonance imaging
M1: primary motor cortex
RCT: randomised controlled trial
tDCS: transcranial direct current stimulation
TMS: transcranial magnetic stimulation
References


