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Optimizing Current and Electrode Montage for Transcranial Direct Current Stimulation in Stroke Patients

Junior Investigator Proposal supporting

MUSC Center of Biomedical Research Excellence (COBRE) Application

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Specific Aims:

Transcranial direct current stimulation (tDCS) is a non-invasive brain stimulation technique [1-4] that can modulate cortical excitability of targeted brain regions. One can measure tDCS induced changes in excitability using a different noninvasive brain stimulation method - transcranial magnetic stimulation (TMS). Various studies have investigated tDCS use in stroke patients with motor impairments (cumulatively about 200 cases) [5-11]. Although these studies are mostly "proof of concept" with small sample size, they do suggest that tDCS may improve motor function. Before a Phase II single-center clinical trial (a R01 application) to assess the efficacy of tDCS for stroke motor recovery is launched by the applicant, two critical scientific questions need to be answered **FIRST** in order to increase the likelihood of success of clinical trial in planning.

What is the optimal current for stroke patients? No systematic investigation has been done to determine the most efficacious current that is both safe and tolerable for stroke patients. There is empirical evidence that tDCS with current up to 2 mA for 20-40 minutes for either single or multiple sessions can safely be administered to healthy subjects [1,3,12]. It is probably safe for stroke patients, but there are no safety guidelines in this population[12]. Animal studies [13,14] have established a much higher safety limit (>50X) than the human protocols, but safety concerns have limited tDCS current to 2 mA for human subjects. This is a critical issue to explore because it is likely that a higher current is more efficacious in increasing cortical excitability and hence motor recovery in stroke patients.

Aim 1: To determine the optimal tDCS current in stroke subjects

The optimal current balances cortical excitability and tolerability/safety in stroke patients. We will invesitigate the optimal curent in range of 1 mA to 4 mA. We hypothesize that 4 mA is tolerable, safe and can induce the highest level of cortical excitability in the lesional motor cortex. We will adapt the classic, gold-standard 3+3 experimental design that is commonly used to find the maximal tolerable dose in drug trials. Tolerability/safety will be investigated by monitoring clinically detectable symptoms by monitoring vital signs and surveying subjects with a quesionnaire before and after stimulation, additionally by detecting subclinical neuronal injury using (1) Magnetic Resonance Imaging/Diffusion Weighted Imaging (MRI/DWI) to detect any focal ischemia; and 2) Molecular biomarkers: S100b and neuron-specific enolase (NSE) to detect neuronal injury. Cortical excitability is measured by the amplitude of motor evoked potentials (MEPs) induced by TMS from the abductor pollicis brevis muscle of the affected side.

What is the optimal tDCS electrode montage for stimulation? According to the theoretical model underlying the use of tDCS for stroke motor recovery, stroke causes an "interhemispheric imbalance [15-17]" with decreased motor excitability in the ipsilesional hemisphere and excessive excitability in the contralesional hemisphere. This has led to studies testing the effects of anodal (excitatory) stimulation to the lesional hemisphere [8,18], cathodal stimulation (inhibitory) to the non-lesional hemisphere [6,11] or combined bi-hemispheric stimulation [19,20]. However, it has not been established which montage can induce the maximal cortical excitability, and eventually the maximal motor recovery.

Aim 2: To determine the optimal tDCS electrode montage in stroke subjects

We hypothesize that the bi-hemispheric stimulation with anodal stimulation on the lesional hemisphere and simultaneous cathodal stimulation on the non-lesional hemisphere induces more cortical excitability in the lesional hemisphere than either anodal stimulation on the affected hemisphere or cathodal stimulation on non-lesional hemisphere alone. We will use a randomized, complete block design in which all stroke subjects undergo each of the three electrode montages (on different days) at the current determined in Aim1. As in Aim 1, MEPs from the affected APB muscle will recorded pre- and post- stimulation to determine the optimal montage for the maximal cortical excitability from the lesional hemisphere.

This proposal lays the scientific foundation for systematic application of tDCS in stroke recovery research. It also serves as an important bridging step between the applicant's prior training and his next milestone - a Phase II single-center clinical trial (a R01 application) to assess the efficacy of tDCS for stroke motor recovery with the optimal current and stimulation montage determined by this proposal.

A: Significance:

A1. Motor Deficits Associated with Stroke: There are 795,000 new strokes each year in the US and stroke is a leading cause of long term disability [21,22]. Motor deficit is the most common deficit after stroke [23]. Stroke recovery strategies, such as, innovative equipment (robotic[24]), cellular therapy[25], and brain stimulation (invasively[26] or noninvasively[27]) seek to enhance neural plasticity thereby improving function. tDCS is a portable, non-invasive brain stimulation technique [1-4] that can be applied simultaneously with rehabilitation therapy with potential to improve motor function in stroke survivors.

A2. tDCS to Augment Motor Improvement: tDCS can modulate the excitability of targeted brain regions by altering neuronal membrane potentials resulting from low-voltage, direct current that is transmitted through the skull via surface electrodes [4]. Behavioral effects after a single 10-40 minute stimulation session have been demonstrated to outlast the stimulation period by as long as 90 minutes [4,28,29]. This is most likely due to the modulation of cellular NMDA-receptors [4,28] and through augmentation of synaptic plasticity that requires the presence of BDNF [30]. To date, tDCS has been tested across the world in thousands of healthy subjects and patients with various disease conditions [12]. In 12 published studies (summaried in applicant's review[31]), mostly "proof of concept" studies with small sample size, subjects have included about 200 stroke patients with motor deficits with current ≤ 2 mA. This technique shows promise of emerging as an adjunctive therapeutic tool to promote motor recovery; however, two scientific questions must be answered before it can be further tested in large phase II/III clinical trial. For example, the optimal current (dose) is not clear, nor is the electrode montage for stroke patients.

A3. Safety and Current (Dose) Issues: Theoretical knowledge regarding the safety and tolerability of electrical stimulation is based on physical aspect of this technique and data from both animal and healthy human studies. These data support the safety of the present tDCS protocols [1,3,4,13,14,32]. Heatlhy subjects are exposed to "no significant risk" at currents ranging of 0.5 to 2 mA for up to 40 minutes either over a single session or multiple sessions. However, safety implications are not clear in stroke patients (i.e., subjects with a brain lesion) and there may be potential for increased risk. So far there has been no single, dedicated study to systematically evaluate the safey and tolerability of tDCS at various currents in the stroke population.

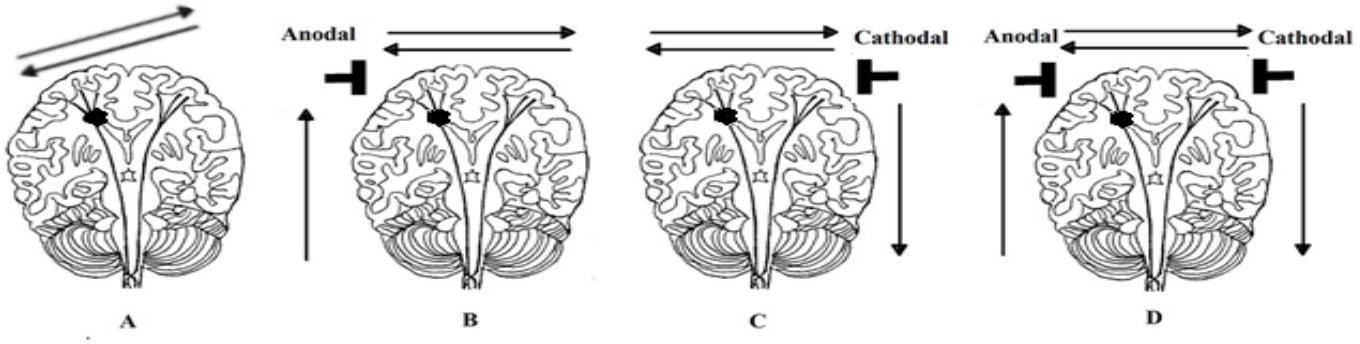
Commonly reported side effects occured at the site where the electrodes are placed and are a minor issue [3,33]: local scalp itching, tingling or burning sensations, transient headaches or stimulation-induced skin lesions [34,35] or contact dermatitis [36]. The size of the eletrode pad also matters in terms of safety and tolerability. Electrode size determines current density. It is defined as current/electrode contact area. Depending on the size of the electrode (typically 3 × 5 cm², 5 × 5 cm^2 or 5 × 7 cm²), current density even at 4 mA – the highest current available for clinical research (Chattanooga Dual-Channel Iontophoresis Device), is approximately 0.11 mA/cm^2 to 0.27 mA/cm^2. This current density is considerably below the safety threshold determined by invasive stimulation technique [32] or the safety limit (~2%) established in an animal study[14]. While underdosing is possible, safety concerns have led researchers to avoid higher tDCS current in stroke patients. Two studies

show that 2 mA is more effective than 1 mA in enhancing cognitive effects in healthy subjects [37] and working memory in patients with Parkinson disease[38]. It is not clear whether there is a linear current (dose) response relationship associated with current and cortical excitability, especially when the current exceeds 2 mA. Patient protocol evolution must balance safety and current (dose). For research with tDCS, it is critically important maximal current levels not be "frozen" at an arbitrary limit of 2 mA, especially if a higher dose is tolerated and produces more excitability changes and are thus likely more effective. Without performing an initial current (dose) finding and safety study, the field risks performing clinical trials that might be ineffective or less effective, but solely because of underdosing. Rather than wasting the time and money on potentially under-dosed studies, it is important to establish this knowledge of dose effects now.

A4. Electrode Montage for Stimulation In Stroke Patients: The second scientific gap concerns the optimal electrode montage that is most favorable for cortical stimulation in stroke patients. The theoretical model [15-17] that serves as the basis for tDCS for post-stroke motor recovery is that of "interhemispheric imbalance **(Figure 1A)**". After a stroke, there is decreased motor activity in the ipsi-lesional hemisphere and excessive activity in the contra-lesional hemisphere, and they are associated with poor motor recovery. In efforts to correct such imbalance, tDCS was applied either by

- Anodal stimulation (**Figure 1B**) on the affected hemisphere [8,18] to up-regulate the cortical excitability, or
- Cathodal stimulation (**Figure 1C**) on the non-lesional hemisphere to down-regulate the cortical excitability [6,11], or
- Bi-hemispheric stimulation (**Figure 1D**) with anodal stimulation on affected hemisphere and simultaneous cathodal stimulation on non-lesional hemisphere [19,20] to up regulate and down regulate cortical excitability simultaneously.

Figure 1. Schematic Model Interhemispheric Imbalance and Montage of Direct Current Stimulation



All three electrode stimulation montages demonstrated various promising improvements in motor functions in stroke patients. Two studies [39,40] with healthy subjects suggest bi-hemispheric stimulation may have advantages over uni-hemispheric stimulation. One TMS study[41] with bihemispheric rTMS suggested that the bi-hemispheric stimulation yielded stronger effects than uni-hemispheric stimulation. It remains unclear which approach is optimal for stroke patients.

This proposal will address those two issues. We aim to solve a much needed scientific issue and to close a scientific gap by establishing safety limits and identifying the optimal current and electrode montage for stroke patients. This project defines the fundamental work needed before systematically investigating the application of the technique in promoting motor recovery in stroke patients, thereby improving the odds of success for the future clinical trial.

B. Innovation:

- **Innovative trial design.** We innovatively adapt a 3+3 trial design **(Figure 4)** that is commonly used in drug trials to assess safety and tolerability of tDCS current for Aim 1. Current associated with tDCS technique is like dosage to a drug that can be adjustable. Evaluating safety/tolerability of tDCS technique is similar to assessing drug safety and dosing finding. While innovative, the design is simple and easy to understand as well as to implement.
- **Use of MR imaging and biomarkers to test for subclinical neuronal injury.** We will test for subclinical injuries with a combination of imaging and biomarker measurements to best ensure thesafety in stroke subjects, as opposed to only assessing safety and tolerability with clinically detectable symptoms as done in most of the previous tDCS safety studies. MRI Diffusion Weight Imaging, dependent on the motion of water molecules, provides information regarding tissue integrity. It is a gold standard for early detection of brain ischemia [42] with a high sensitivity as well as high specificity [43]. An Apparent Diffusion Coefficient (ADC) value decrease represents diffusion restriction while an ADC value increase indicates an increase in water mobility in the stimulated cortex. Neuron-specific enolase (NSE) and S-100b protein (S-100b) are two widely investigated molecular markers for brain injury [44-46]. NSE originates from the cytoplasm of neurons and neuroendocrine cells. S-100 protein is a dimeric acidic calcium-binding protein found in the brain with high concentrations in astrocytes and Schwann cells. The serum levels of these proteins are elevated after different types of brain injury including focal and global ischemia [46-48], traumatic brain injury [49] and hypoxic brain injury [50].

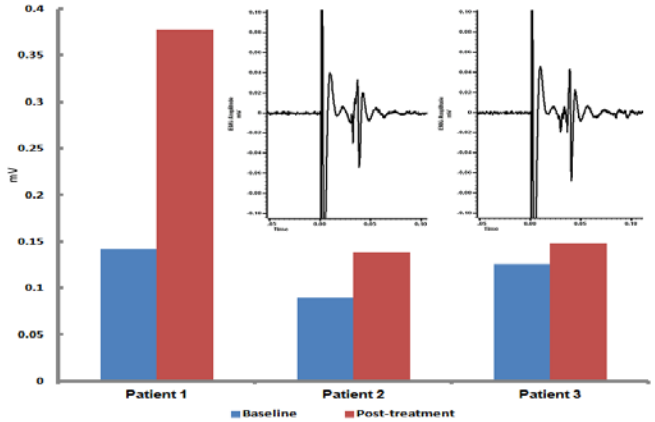
C: Preliminary data:

Table 1. Safety Profiles with Current Intensity at 2mA

Items	Patient 1	Patient 2	Patient 3
Headache	(+)	(-)	(-)
Scalp pain	(-)	(-)	(-)
Tingling	(+)	(-)	(-)
Itching	(-)	(-)	(-)
Burning	(-)	(-)	(-)
Electric Shock	(-)	(-)	(-)
Sleepiness	(-)	(-)	(-)
Trouble Concentrating	(-)	(-)	(-)
Mood Changes	(-)	(-)	(-)
Skin Redness	(-)	(-)	(+)
Others	(-)	(-)	(-)
Vitals Signs	Stable	Stable	stable
MRI Scan (Visual inspection)	No new lesion	No new lesion	No new lesion
MRI Scan (ADC)	No changes	No changes	No changes

minutes is safe and tolerable in a single session as well as ten sessions over 2 weeks. One subject experienced transient mild headache and tingling

Figure 3: MEPs Changes Between Baseline and Post-Treatment



In the applicant's lab, a pilot study "*Bihemiespheric Stimulation and Constrainted-Induced ovement Therapy (CIMT) to Promote Post-Stroke Motor Recovery*" is currently ongoing with 3 stroke subjects who have completed the protocol. While the main objective of the study is to determine whether tDCS can amplify the effects of CIMT, the study also provides a test of the safety and tolerability of stimulation current at 2 mA. To date, we have demonstrated that bihemispheric stimulation with 2 mA and electrode size of 15 cm² (current density is 0.133mA/cm²) for 30

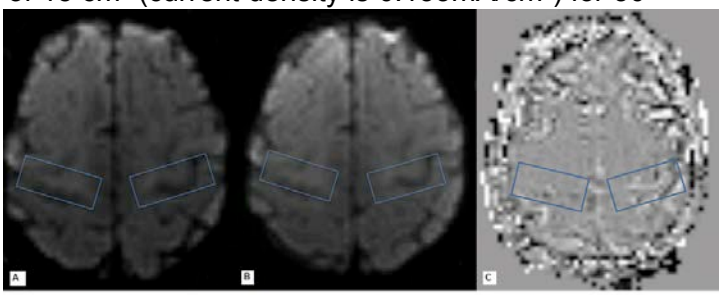


Figure 2: An Example of One study Subject (A): DWI Sequence Pre-stimulation; (B): DWI Sequence Post-Stimulation; (C): ADC Subtraction Map (Pre and Post) of same slice.

during stimulation, and another subject experienced transient scalp redness under the anodal stimulation

electrode. Vital signs for all four patients were stable and no one discontinued the study due to side effects **(Table 1).** No new focal ischemic lesions were observed on MRI DWI sequence and no changes in Apparent Diffusion Coefficient (ADC) map was observed in any of 3 subjects (**Figure 2**) .

We also observed an increased cortical excitability on the affected motor cortex in all three stroke subjects **(Figure 3)**. The increased cortical excitability from lesional motor cortex is associated with postive changes with behavioral assessment using FM-UE scal and Wolf Motor Functional Test (data not shown).
In summary, consisent with prior studies and consensus from experts in the field, 2 mA appears to be a safe current for stroke patients. Further, we have established the feasibility of recruiting stroke subjects and using tDCS as proposed and using TMS for cortical excitability assessment.

D: Approach:

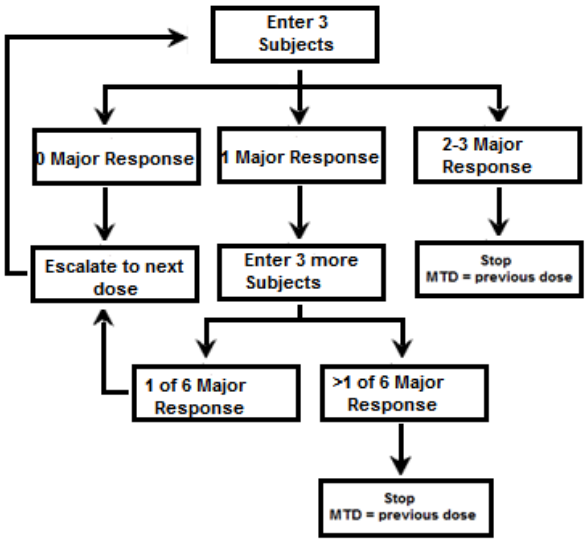
This grant proposal is divided into two independent, but well-connected experiments – Aim 1 to determine the optimal *current* and Aim 2 to address the *optimal stimulation montage.*

Aim 1: To determine the optimal current in stroke patients

D1.1 Design:

This aim is a phase I type study to determine the most efficacious current for tDCS, recognizing that this current level must be tolerable and safe. The trial design is adapted from the classical, gold-standard 3+3 drug trial design [51,52], which is commonly used to find the maximal tolerable dose for a chemical drug. The main justification for this design is that the maximal tolerable current (dose) is usually and arbitrarily defined as the dose at which one-third of the patients experience current (dose)-limiting major side effects/response. The algorithm for current (dose) escalation is detailed in (**Figure 4)**. The study starts with 1 mA : (a) if no major response occurs in any of the three subjects, a next current at 2 mA will be used next; (b) if major response occurs in 2-3 study subjects, then the trial will stop at this current level; (c) if major response occurs in one subject, then 3 additional study subjects will be entered to further test the safety of same current dose; if no major response occurs, then the current will be escalated next current level; if further major response occurs, then the trial will terminate. The previous current level will be defined as the maximal tolerable current (dose). The current tDCS device avaiable on the market (Chattanooga Dual-Channel Iontophoresis Device) for research use has a maximal current of 4.0 mA. The sequence for current escalation will be 1.0 mA >> 2.0 mA >> 2.5 mA >> 3.0 mA >> 3.5 mA >> 4.0 mA.

Figure 4: 3 + 3 Design



While we vary current in this protocol, other features of the stimulation will be controlled:

- **Duration of stimulation will be 30 minutes:** Data show the increased excitability of tDCS can last as long as 90 minutes. Our rehabilitation intervention will take one hour. Thus, by using 30 minutes of

stimulation, as has been commonly used in stroke studies, we should ensure that the potentially beneficial effects of the stimulation will not wear off during the course of rehabilitation.

- **Size of electrode will be 3 × 5 cm²:** The commonly used 3 × 5 cm² size pad is chosen because it is adequate to cover the the primary motor cortex – our area of interest for brain stimulation in stroke subjects to facilitate upper extremity motor function recovery.
- **Electrode montage will be bi-hemispheric stimulation :** the bi-hemispheric stimulation montage is used for Aim 1 because safety profiles can be evaluted for both anodal stimulation and cathodal stimuation on the same study subject.

Definition of Major Response and "Stopping Rule":

A major response is defined by any of the following serious adverse events occurring during the study period. It serves as the "stopping rule" as detailed in the **Figure 4** above.

- Second degree scalp burn at the site of electrode pad; or
- Seizure; or
- New lesion(s) on DWI sequence of MRI scan and the lesion(s) is not explained by any other cause(s) or decreased ADC under the electrode stimulating motor cortex area; or
- Patient discontinues from the study due to any reasons above.

Inclusion Criteria:

Each subject must meet **all** of the following criteria to participate in this study.

- 18-80 years old with a first-ever ischemic stroke that occurred at least 6 months ago;
- Finished rehabilitation therapy(including inpatient or outpatient PT/OT/SP) at least one month ago;
- Unilateral limb weakness with Fugl Meyer-Upper Extremity Scale score less than or equal to 58 (out of 66);
- MEPs is inducible on abductor pollicis brevis (APB) muscle on the affected side by TMS.

Exclusion Criteria:

Subject who meets **any** of the following criteria will be excluded from the study.

- Primary intracerebral hematoma, or subarachnoid hemorrhage,
- Bihemispheric ischemic strokes;
- History of prior stroke or old infarct demonstrated on the CT or MRI or documented in medical records;
- Other concomitant neurological disorders affecting upper extremity motor function;
- Documented history of dementia before or after stroke;
- Documented history of uncontrolled depression or psychiatric disorder either before or after stroke which could affect their ability to participate in the experiment;
- Uncontrolled hypertension despite treatment, specifically SBP/DBP>=180/100mmHg at baseline;
- Presence of any MRI/tDCS/TMS risk factors: a) an electrically, magnetically or mechanically activated metal or nonmetal implant including cardiac pacemaker, intracerebral vascular clips or any other electrically sensitive support system; b) non-fixed metal in any part of the body, including a previous metallic injury to eye; c) pregnancy, since the effect of tDCS on the fetus is unknown; d) history of seizure disorder or post-stroke seizure; e) preexisting scalp lesion, bone defect or hemicraniectomy.

D1. 2. Recruitment Process & Procedure:

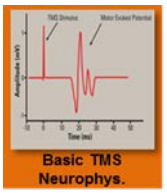
A schedule of assessments is presented in **Table 2.**

Table 2	Baseline*	Stimulation Day (Day 0)	Follow-up

	(Day –7 ± 7)	Pre-stim	Post-stim	(Day 14 ± 4)
Eligibility Determination and Informed Consent	X			
Demographics	X			
Vital Signs and Safety Questionnaire	X	X	X	X
TMS Evaluation	X	X	X	
Laboratory		X	X	X
Brain MRI		X	X	

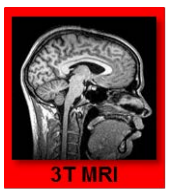
* Baseline can be same day as Stimulation Day to facilitate recruitment.

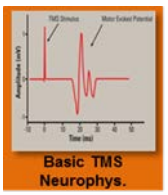
- **<u>Baseline Day (Total Estimated Time: ~ 2 hours)</u>**
  1. Subject reports to the College of Health Professions (CHP) building QBAR laboratory.
  2. Subject is consented first, then starts screening process.
  3. Vital signs are taken. Urine pregnancy test for subjects of childbearing age. Estimated time is 10 minutes.
  4. Demographics collected, including age, ethnicity, gender, time from stroke (in months), side of infarct, handedness, medical history and medication list. Estimated time is 20 minutes.
  5. A technician from the **BSTIM Core** will perform a ***Basic TMS Neurophysiological Assessment*** to determine if a MEP is inducible on abductor pollicis brevis (APB) muscle on the affected side by TMS (Details in **BSTIM Core**). Estimated time is 30 minutes.

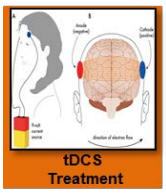


  6. Subject takes structured questionnaire. The questionnaire inquires whether the patient has experienced any of the following symptoms: headache, nausea, burning, tingling, itching, neck pain, electric shock, inattention and etc. It also has open questions inquiring about subject's general impression about the stimulation. Estimated time is 10 minutes.
  7. A therapist from the **QBAR Core** will perform ***Clinical Assessments*** including upper-extremity Fugl-Meyer (UE-FM) scale. Estimated time is 30 minutes.
  8. Subjects who meet the inclusion/exclusion criteria are eligible for the study and move to the next step.

- **<u>Stimulation Day (7 days after baseline day; Estimated Time: ~4 hours)</u>**
  9. Subject report to Center of Advance Imaging for Research (CAIR). The **NI Core** will perform ***structural 3T MRI*** (Details in **NI Core and MRI protocol**). Estimated time is 30 minutes.
  10. Subject is transported to CHP research building QBAR laboratory (less than 100 yards away).
  11. Blood draw is performed collecting 10 ml's and specimen is sent to Dr. Yushing Zhu's laboratory in the Children's Hospital. . Urine pregnancy test for subjects of childbearing age. We have updated the protocol. Estimated time is 10 minutes.
  12. Subject takes structured questionnaire survey. Estimated time is 10 minutes.
  13. A technician from the **BSTIM Core** will perform a ***Basic TMS Neurophysiological***



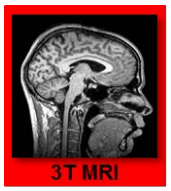




Assessment to determine cortical excitability (details in **BSTIM Core and TMS protocol**) in the BSTIM laboratory in the CHP research building (the room next door to the QBAR laboratory). Estimated time is 30 minutes.

14. Vital signs are taken. Estimated time is 5 minutes.
15. The **BSTIM core** oversees tDCS stimulation protocol. The PI will administer the tDCS under the supervision of the **BSTIM core**.
16. Coincident with the beginning of stimulation, the therapist from the **QBAR Core** begins a one hour ***Upper Extremity Rehabilitation*** session of patient-targeted upper extremity function therapy (details in **QBAR Core and Therapy Protocol**). Estimated time is 60 minutes.

17. At the end of the rehabilitation session, the technician from the **BSTIM Core** will again perform a ***Basic TMS Neurophysiological Assessment*** to determine cortical excitability. Estimated time is 30 minutes.
18. The structured questionnaire survey is performed again. Estimated time is 10 minutes.
19. A second blood draw is performed and specimen is delivered to Dr. Yushing Zhu's laboratory in the Children's Hospital I. Estimated time is 10 minutes.



20. Subject is transported to CAIR. **NI Core** will again perform ***structural 3T MRI*** (Details in **NI Core and MRI protocol)**. Estimated time is 30 minutes.
21. Patients are advised to call the study team if they experience any untoward side effect before the follow up day.

- **<u>Follow-up day (14 days after stimulation day; Estimated time: ~ 0.5 hour)</u>**

22. Subject reports to CHP research building QBAR laboratory.
23. A blood draw is performed collecting 10 ml's and specimen is sent to Dr. Yushing Zhu's laboratory in the Children's Hospital. Estimated time is 10 minutes.
24. The structured questionnaire survey is performed again. Estimated time is 10 minutes.

- **<u>Final analyses:</u>**

25. Note that these analyses must be completed for each set of 3 subjects in a group to determine whether one or more major responses occurred before enrollment continues. If no major response occurs, the next 3 subjects will be entered to go through procedure 9-23 according to **Figure 4.**

<u>D1. 3. Statistical Analysis:</u>

Due to the nature of 3+3 design (**Figure 4**), the theorectical sample size varies from 3 to 36 depends on when the trial stops under "stopping rule". However, we hypotheize that no major responses will occur at any current (dose) level and the current will be escalate to 4 mA, thus we predict that 18 stroke subjects will be required for Aim 1. First, patients' demographic and clinical characteristics will be summarized. Summary statistics for continuous variables includes number of subjects, means, median and standard deviations. Summary statistics for discrete variables includes counts and percentages for each category. Secondly, the safety measures will be summarized by current(dose) group with descriptive statistics. Thirdly, a current(dose) response curve will be plotted by MEPs amplitude versus current (dose) groups. Per DSMB recommendation, S-100 and NSE will not used as a stopping rule, however, they will be still collected and analysed separately and presented to DSMB meeting.The statistical analyses will be performed with SAS V9.2 (SAS Institute Inc., Cary NC).

<u>D1. 4. Anticipated Results:</u>

We anticipate that 4.0 mA will be tolerable and safe both at clinical and subclinical levels, as evidenced by a lack of major responses in the study subjects. In addition we expect that 4.0 mA will produce the largest increase in MEPs amplitude compared with other current levels.

Aim 2: To determine the optimal stimulation montage in stroke patients

D2.1. Design:

This is a repeated measures design in which a group of stroke subjects will each receive one of three stimulation montages (anodal stimulation, cathodal stimulation and bihemispheric stimulation) on three different days (separated by at least two days in between). The order in which they receive the three montages will be randomized to account for order effects. The primary purpose here is to determine the stimulation montage that induces the largest cortical excitability on the lesional hemisphere**.** The rationale of the design is that each subject serves as his/her own control, thus eliminating between-subject variation and makes it easier to determine changes in cortical excitability between conditions. We will require at least two days between each stimulation session so that there is little risk of carry over as the after-effect from tDCS have been shown to last over one hour[29].

C2.2. Recruitment Process & Procedure:

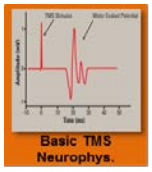
We will use the same study population as in Aim 1, defined by the same inclusion and exclusion criteria, for Aim 2. The same TMS, tDCS and Rehabilitation Protocols from Aim 1 will be applied to Aim 2. Those subjects who completed Aim 1 are still eligible to be enrolled in Aim 2. The subjects, therapist and assessor are all blinded to the condition.

A schedule of assessments is detailed in the following **Table 3**.

Table 3	Baseline* (Day –7 ± 7)	Session I (Day 0)		Session II (Day 2)		Session III (Day 4)	
		Pre	Post	Pre	Post	Pre	Post
Eligibility Determination and Informed Consent	X						
Demographics	X						
Vital Signs and Safety Questionnaire	X	X	X	X	X	X	X
TMS Evaluation	X	X	X	X	X	X	X

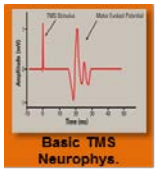
* Baseline can be same day as Session I to facilitate recruitment.

Baseline Day (Total Estimated Time: ~ 1.5 hour)



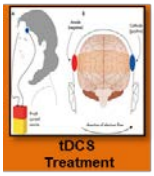
1. Subject reports to the CHP building QBAR laboratory.
2. Subject gets consented first and starts screening process.
3. Demographics collected, including age, ethnicity, gender, time from stroke, side of infarct, handedness, medical history and medication list. Estimated time is 20 minutes.

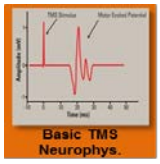
4. Subjects of childbearing age will have a urine pregnancy test.
5. A technician from the **BSTIM Core** will perform a ***Basic TMS Neurophysiological Assessment*** to determine if a MEP is inducible on abductor pollicis brevis (APB) muscle on the affected side by TMS (Details in **BSTIM Core**). Estimated time is 30 minutes.
6. A therapist from the **QBAR Core** will perform ***Clinical Assessments*** including upper-extremity Fugl-Meyer (UE-FM) scale. Estimated time is 30 minutes.
7. Subjects who meet the inclusion/exclusion criteria are eligible for study and move to the next step.



Stimulation Day (Total Estimated Time: 2.5 hours)

8. Subject reports to the College of Health Professions (CHP) building QBAR laboratory. Subjects of childbearing age will have a urine pregnancy test prior to each stimulation session.
9. The **BSTIM core** will perform cortical excitability assessment using TMS in the BSTIM laboratory in the CHP research building (the next room from the QBAR laboratory). Estimated time is 30 minutes.
10. The **BSTIM core** oversees tDCS stimulation protocol. The PI will administer the tDCS under the supervision of the **BSTIM core**.
11. Coincident with the beginning of stimulation, the **QBAR core** begins a one hour rehabilitation session of patient-targeted upper extremity function therapy. Estimated time is 60 minutes.
12. At the end of the rehabilitation session, **BSTIM core** will again perform cortical excitability assessment using TMS. Estimated time is 30 minutes.
13. Subject will go home and rest for 2 days, then come back to repeat the procedure 7-11 twice to received other two stimulation sessions.





D2.3 Statistical Analysis:

The primary outcome measure will be the quotient of post/pre MEPs amplitude recorded from the affected APB muscle to represent the cortical excitability in the lesional motor cortex. Assuming power of 85% and type I error of 0.05, (Bonferroni adjusted for 3 comparisons), 18 subjects would be sufficient to detect an effect size of 0.85 of the MEPs between two treatments groups using a paired t-test. We will summarize the demographic and clinical characteristics of the sample using means and proportions. Although the study is powered based on paired t-tests, we will analyze the data using a repeated measures ANOVA for the post/pre MEP quotient and include covariates in the secondary analyses to glean more information about the variability in the outcome. The statistical analyses will be performed with SAS V9.2 (SAS Institute Inc., Cary NC).

D2.4 Anticipated Results:

We anticipate that the bihemispheric stimulation (anodal on the lesional hemisphere and cathodal stimulation on the non-lesional hemisphere) will induce the largest increase in the cortical excitability in the lesional hemisphere compared with the other two stimulation montages – anodal stimulation on the lesional hemisphere only or cathodal stimulation on non-lesional hemisphere only.

D3.1 Limitation, Potential Pitfall and Alternative Approach:

- Why not perform these studies in healthy subjects first? We are pursuing a similar study in healthy subjects through other funding mechanisms. However, the information from healthy subjects can not be applied to stroke patients because they are completely different populations. In general, there is a concern for potential increased risk for stroke patients for tDCS stimulation.
- We are testing for effects and safety with a single session, but the treatment studies would likely involve multiple tDCS sessions? Feasibility, unlikely to see cumulative safety issues over multiple sessions.
- If the difference of induced cortical excitability between different electrode stimulation montage is smaller than expected, the alternative approach is to increase sample size or consider multiple sessions rather than single session.

D4. 1 List of Protocols:

MRI Protocol:

Study subjects will be scanned at the Center of Advanced Imaging (**NI core**) by a Siemens 3T Trio MRI scanner using a standard radiofrequency head coil at before and within 2 hours after stimulation. During MRI, electrode positions will be marked by placing vitamin pills detectable in MRI scans at the center of the tDCS electrodes. Areas of 3* 5 cm, which were the size of the tDCS electrodes, will be determined as regions of interest (ROI) around this center for the stimulated primary motor cortex. Head motion will be minimized by foam padding and forehead-restraining straps if necessary. T1 contrast enhanced weighted anatomical image (IR/TR/TE = 1100/1900/3.37; resolution = 1 x 1 x 1 mm^3; FOV = 256 x 256), Fluid attenuated inversion recovery (FLAIR, TR/TE = 9000/89; resolution = 0.43 x 0.43 x 5 mm^3; FOV = 220 x 192.5) and DWI (TR/TE = 5.5 s/99 ms; resolution = 3 x 3 x 3 mm^3; FOV = 222 x 222). In T1- diffusion-weighted studies, and ADC maps will be visually inspected for signal changes by a blinded board certified neuro-radiologist or neurologist. Furthermore, pixel by-pixel subtraction of the post-stimulation ADC parametric images from the baseline ADC parametric images will be performed by means of an implemented scanner software algorithm for improved differentiation and highlighting of possible locally restricted ADC changes. Statistical difference between the ADC values before and after stimulation will be calculated and analyzed.

Laboratory Protocol:

The laboratory test will be processed and analyzed at Dr. Yushing Zhu's lan in the Children's Hospital The serum samples will be collected at baseline, before stimulation, after stimulation and one week after stimulation. The samples will be centrifuged at 3000 rpm for 10 min and stored at −20 °C prior to the analyses. The NSE assays will be performed using a radioimmunoassay technique (Cobas Core NSE EIA, Hoffmann-La Roche, Switzerland). The S-100b concentrations will be measured by an immunoluminometric assay for the quantification of protein (LIAmat® Sangtec®100, Sangtec Medical, Sweden). The sensitivity of the S-100b assay will be b0.02 μg/l.

TMS protocol:

The TMS evaluation will be conducted at the Brain Stimulation Laboratory (**BSTIM Core**) and performed by trained research staff members who are blinded to the study. Subjects will be seated comfortably in a chair specifically designed with a chin rest, head rest and molded armrest in which they could keep their arms in a constant relaxed position. Subjects have 9mm Ag/AgCl surface electrodes placed over the abductor pollicis brevis (APB) muscle. The TMS coil will be positioned using a neuronavigational guidance system (Brainsight ™, Rogue Research Inc., Montreal Quebec) along with the participant's MRI. Given that neuroanatomy is often shifted post stroke, this system will allow us to maximize precision of the coil placement. The stereotactic guidance system consisted of trackers attached to the TMS coil and to the subject's head which could be detected by an optical position sensor. Following a calibration procedure, these trackers are monitored by the

software which displayed the position and orientation of the coil on the subject's anatomical MRI scan. This technique facilitates repeated positioning of the TMS coil over the subject's brain. The location of the motor area governing the thumb is isolated for each individual by moving the TMS coil in 0.5 cm steps to find the site at which the maximum twitch could be elicited. EMG signals are recorded using a closed-loop system, in which feedback from the EMG is used to control the output of the TMS machine via specially written scripts (Spike^2 software, Cambridge Electronic Design, Cambridge, UK). The raw EMG signal is amplified and bandpass filtered (low-pass = 0.5 Hz, high-pass = 1 kHz) using the CED 1902 Quad-System (Cambridge Electronic Design Limited, Cambridge, U.K.). It is digitized (sampling rate = 5 kHz) and recorded onto a PC using Spike^2 software and a Micro 1401 data acquisition unit (Cambridge Electronic Design Limited, Cambridge, U.K.). The resting motor threshold (rMT) is determined using a procedure known as the maximum-likelihood strategy in which the parameter estimation by sequential testing (PEST) algorithm is used [53,54]. The PEST method uses the EMG response to the TMS pulse to determine if TMS intensity needs to be increased or decreased. In our closed-loop system, the TMS machine is automatically adjusted according to PEST output, resulting in an MEP of 50 μV in 50% of trials. Thus, the rMT is determined in a matter of minutes, is much less labor intensive compared with the Mills-Nithi IFCN method and less prone to human error. Cortical excitability was tested by delivering 10 TMS pulses to M1 of each hemisphere (TMS intensity of 20% above the individual rMT to induce MEPs of at least 1 mV, if MEPs size is smaller than 1mV, then TMS intensity will go up to 100% and kept at 100%). The same intensity was kept constant pre and post-stimulation. Ten MEPs will be recorded for each hemisphere, and their peak-to-peak amplitude will be averaged to determine MEPs amplitude.

tDCS Protocol:

Direct current stimulation will be delivered to the study subjects through 2 saline-soaked EasyPad (3cm * 5cm=15cm²) using a Chattanooga Dual-Channel Iontophoresis Device (Chattanooga Medical. Inc). For this protocol, the bihemispheric stimulation montage will be used because a safety profile can be evaluated for both anodal stimulation and cathodal stimuation on the same subject. The anodal stimulation will be placed over the ipsi-lesional and the cathodal over the contra-lesional motor cortex (C3 and C4 areas by using the international 10 –20 EEG electrode systems). Electrode pads will be disposed after each stimulation session. During the middle of stimulation, an inspection of electrode pads will be conducted to make sure they have not dried out, and the scalp temperature will be measured using a touchless thermometer. Stimulation will last for 30 minutes. Thus, subjects will perform for thirty minutes with stimulation and 30 minutes after it has ended. The QBAR core therapist will be blinded to dosage and montage of stimulation by the BSTIM core technician. The experiment will be conducted in the Upper Extremity Funciton Laboratory of the Center for Rehabilitional Research in Neurological Conditons (**QBAR core**).

Customized Task-Oriented Training Protocol:

Subjects will engage in one hour of concentrated task-practice directed at the paretic arm and hand. Task-practice sessions will be structured and supervised by a trained and licensed therapist. Selection of functional motor tasks for a given subject will be derived from their baseline FM-UE score [55]. Based upon assessment information obtained from the baseline FM-UE score, motor tasks will be selected that are optimally challenging and customized to personal abilities and goals. This method also allows for systematic progression of task types and difficulty to maintain a "just-right" match between the subject's abilities and task-difficulty. Tasks practices will address specific gross-motor, fine-motor, proximal, and/or distal movement components within the context of functional tasks. Repetition and feedback schedules and methods will follow the theoretical framework of the Task-Oriented Approach.

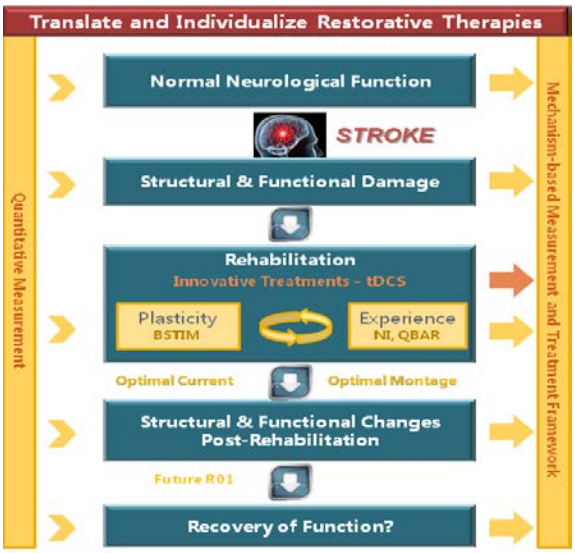
D5. 1. Timeline:

About 600 patients per year with a primary diagnosis of acute ischemic stroke were admitted to MUSC Stroke Center. About 12-15% patients (6 - 8 patients per month) can meet the inclusion criteria. In addition, Center of rehabilitation Science in Neurological Conditions mains a database with 50 stroke subjects who were agreed to be contacted for future research studies, this study will also recruit from the Registry for Stroke Recovery (RESTORE-Pro#00037803), which is a registry with subjects consented for future contact. (please refer to details in D5.5). Thus, we should have no problem obtaining the required number of subjects from the **CTTR Core**. The protocol will be submitted to the MUSC IRB with expectation of final approval by June, 2013. Subject enrollment will begin immediately if the proposal is funded. We expect to complete the Aim1 in year 1 and aim 2 in year 2 (**Table 1).**

Table 1	**Pre-year**	**Year 1**				**Year 2**			
Quarter	**1**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Research Plan	Receive IRB Approval	Start recruitment for Aim1, and plan to recruit 2-3 subjects per months, complete Aim1 by the end of year 1.				Start recruitment for Aim2, and plan to recruit 2 subjects per months and complete it at the end of year 2			Start R01 proposal
Training Classes		Clinical Trial Methods Course in Neurology				Imaging tool analysis in FSL and SPM			
Scientific Meetings		Present data from Aim1 internally - Neuroscience Grand Rounds; - SCTR SOCRATES club - Monthly stroke team meetings;			International stroke conference or American Society of Neuro-rehabilitation Conference	Present data from Aim2 internally - Neuroscience Grand Rounds; - SCTR SOCRATES club - Monthly stroke team meetings;			
Publications		Manuscript#1: efficacy and safety of various current level of tDCS in stroke patients				Manuscript # 2: Optimal tDCS stimulation montage for stroke patients			

D5. 2. Integration with COBRE:

This proposal is well integrated into the COBRE application. It utilizes all of three scientific cores in the COBRE (**Figure**), and most importantly, the applicant has a clear plan for a next project to continuously utilize all of cores. Further, the proposed study will advance the **BSTIM Core** as it potentially leads to a new treatment for post-stroke motor recovery. Specifically, much of the study will be performed in the **QBAR Core** laboratories and a QBAR therapist will conduct the therapy session for the experiments outlined in Aim 1 and Aim 2. The **BSTIM Core** will provide the tDCS device, TMS and necessary training. The research assistant from Brain stimulation laboratory, along with the applicant; will conduct TMS assessment of cortical excitability on stroke subjects. The MR scan will be conducted at the Center of Advance Imaging Center (CAIR) where **NI Core** is located. The **NI Core** will also provide necessary training and staff for imaging process and analysis.



The applicant already had multidisciplinary collaboration with other junior investigators in the COBRE application. For example, Dr. Feng has an established collaborative relationship with Dr. Hanlon whose expertise is neuro-imaging with functional MRI and cortical excitability assessment with TMS. They have collaborated on the ongoing pilot study "*Bihemiespheric Stimulation and Constrainted-Induced Movement Therapy to Promote Post-Stroke Motor Recovery".* Part of data from this

study were used as preliminary data supporting two proposals for this COBRE application. Dr. Hanlon's current project also provide a neurobiological basis for stroke recovery. The result from her project could be helpful for Dr. Feng to design the R01 project in planning to uncover the recovery mechanism associated with tDCS. Dr. Feng also has collaborated with Dr. Malcolm whose expertise is investigating rTMS as an adjunct tool for stroke recovery while Dr. Feng's is studying how tDCS promote stroke recovery. tDCS and rTMS are two similar but different non-invasive brain stimulation techniques, they both have the capabilitlity to modulate cortical excitability and induce subsequent brain plasticity, currently they both hold promise for stroke motor recovery. They have been meeting regularly to trouble shoot technical aspects of the two techniques, and working together testing the new magstim® rTMS with brainsight.

D5.3. Mentoring Plan:

Dr. Robert Adams is the **Primary Mentor.** He is a Professor, endowed chair and director of MUSC Stroke Center. His clinical research has involved selection of imaging methods, such as Transcranial Doppler (TCD) and MRI, to assess the intracranial arteries and help predict prognosis in patients with sickle cell vasculopathy. He is an expert in stroke trial design and execution of multicenter studies. He has continuous NIH funding since 1988, and his current portfolio includes a R01, one of three formal "Multiple PI's" in a large U01 clinical trial, and he is the head of a stroke project funded by the Department of Defense. Dr. Adams has substantial experience in nurturing junior faculty throughout his career, including one who has become Chair of Neurology in an academic institution. Since he has been at MUSC in 2007, he has trained two stroke fellows and mentored two junior faculty members who have K award in good standing. Thus, he is the ideal mentor for me as I aim to become a successful academic faculty member. Specifically he will supervise me on clinical trial design and execution and DSMB.

Dr. Mark George is a **Co-mentor.** Dr. George is professor of Department of Behavioral Science and Psychiatry, director of brain stimulation laboratory, an internationally-recognized expert in Non-invasive brain stimulation techniques, including TMS and tDCS. He is one of the founders of therapeutic rTMS techniques, and Editor-in-Chief of the journal *Brain Stimulation.* He has extensive research experience with development and application tDCS in various disease conditions. He will specifically provide mentorship relating to tDCS application and trouble shoot during the experiments.

In addition, I will also receive mentorship from the scientific cores through participation in all of their mentoring activities. By the end of the proposed project, my specific goals are to gain knowledge and be expert in training in trial design, Neuroimaging, and brain stimulation, in addition, I would like to transition into an independent, successful federally funded research scientist in stroke recovery and rehabilitation.

D5.4. Applicant's Future Project (Including R01):

This proposal serves as a critic bridging step between the applicant's prior training and his next milestone - an R01 application. The R01 is planned to submit in the end of year two when the proposed experiments are close to completion. The proposed R01 project is a Phase II single-center clinical trial to assess the efficacy of tDCS for post-stroke motor recovery with the current and stimulation montage determined from this proposal. If R01 project show promising result, Dr. Feng hope to launch a U01 project to further test the tDCS in post-stroke motor recovery in a multi-center phase III clinical trial.

D5.5 Additional information about recruiting patients from research database:

Subjects with stroke will be recruited from the recruitment database maintained at the MUSC Center for Rehabilitation Research in Neurological Conditions (CRRND) which is housed in the CHP Department of Health Sciences and Research (secondary appointment of the PI and primary appointment of the research therapist). The research database (approved MUSC IRB #15991) currently contains ~200 persons with stroke who have signed informed consent and agreed to be contacted for research participation. The research

database is managed by a designated coordinator. This coordinator will be provided with the inclusion/exclusion criteria for the current study. This study will also recruit from the Registry for Stroke Recovery (RESTORE-Pro#00037803), which is a registry with subjects consented for future contact. RESTORE staff will query the registry for potential subjects and provide the Principal Investigator (PI) with the contact information of subjects who meet their criteria. The PI or research staff will contact subject to further screen for potential enrollment. When a potential participant is identified that meet inclusion criteria the PI, project coordinator or research therapist will contact participants by phone to determine if they want to participate in the current study. If they wish to participate they will be scheduled for a time for the PI, project coordinator or research therapist to obtain informed consent for the study proposed. Consent will be obtained after reviewing the protocol and consent form with each potential subject. If a potential subject is deemed unable to participate in informed consent, consent will be obtained from a relative with the patient present. For patients consenting themselves, every attempt will be made to have a relative present during the informed consent. The MUSC IRB will approve the informed consent form.

D5.6 Sharing Data:

If the subject agrees, the data collected and generated from this study will be shared to the Registry for Stroke Recovery (RESTORE-Pro#00037803) by the subject's registry ID. Sharing data from this study with the registry will allow for more targeted recruitment efforts in the future and allow researchers at MUSC to have a more complete registry with key stroke recovery elements including common data and physical function characteristics that are applicable to multiple studies. MUSC researchers and collaborating facilities will be able to query data sets to learn more about recovery of subjects after their stroke through institutionally managed secure servers that will assure HIPAA privacy and security compliance.

Appendix:

1. Total enrollment table

Please refer to statistical analytic plan for sample size calculation, 36 subjects (18 subjects for part I and another 18 subjects for part II) are needed.

TARGETED/PLANNED ENROLLMENT: Number of Subjects			
Ethnic Category	Sex/Gender		
	Females	Males	Total
Hispanic or Latino	2	2	4
Not Hispanic or Latino	16	16	32
Ethnic Category: Total of All Subjects*			36
Racial Categories			
American Indian/Alaska Native	1	1	2
Asian	1	1	2
Native Hawaiian or Other Pacific Islander	0	0	0
Black or African American	8	8	16
White	8	8	16
Racial Categories: Total of All Subjects*	18	18	36

2) Protection to Human Subjects:

Potential Risk to Human Subjects

tDCS: Extensive animal and human evidence and theoretical knowledge indicate that the presently used tDCS protocols up to 2mA are safe[1,3,4]. It involves minimal risk to human subjects. Commonly reported side effects are as followed: skin irritation with itching,tingling, burning sensation, transient headache or nausea. For part one of the study where the level of current is being tested higher than 2mA, there maybe potential side effects. They include burns under the pads, seizure, changes on MRI, and changes in blood test results. If patient experience any of serious adverse event which meet the 'stopping rule", the trial will be evaluted by DSMB and make further decision. The risks associated with pregnancy and tDCS are unknown. Pregnant women are excluded. All female subjects that are of childbearing age that have not had a hysterectomy will be ask their method of birth control and will be questioned prior to each session if there is a possibility that they might be pregnant. Acceptable forms of include birth control condom use or hormonal contraceptives.

MRI: The test will be done using equipment similar to that used for clinical MRI. The current revision of the US Food and Drug Administration guidelines (U.S. Department of Health and Human Services, Food and Drug Administration, Center for Devices and Radiological Health, Guidance for the submission of premarket

notifications for magnetic resonance diagnostic devices, Washington, DC. November 14, 1998) applies to this study. These guidelines state that magnetic resonance imaging systems with main static magnetic field strengths of 4.0T (a measure of magnetic strength) and less, such as the ones used for this study, can qualify as non-significant risk devices.

Because the MRI machine acts like a large magnet, it could move iron-containing objects in the MRI room during your examination, which could in the process possibly harm you. Precautions have been taken to prevent such an event from happening; loose metal objects, like key chains, are not allowed in the MRI room. If you have a piece of metal in your body, such as a fragment in your eye, aneurysm clips, ear implants, spinal nerve stimulators, or a pacemaker, you will not be allowed into the MRI room and cannot have a MRI. Having a MRI may mean some added discomfort to you. In particular, you may be bothered by feelings of claustrophobia and by the loud banging noise during the study. Temporary hearing loss has been reported from the loud noise. This is why you will be asked to wear earplugs. At times during the test, you may be asked not to swallow for a while, which can be uncomfortable.

Venipuncture: The risks of drawing blood include temporary discomfort from the needle stick, bruising and infection. Fainting could occur. There will be a total of 30 ml's collected for the 3 blood draws (10 ml's per draw).

TMS: The TMS coil makes noise, much like a loud pop when it produces its magnetic energy. You may or may not feel your thumb twitch depending on the strength of the TMS pulse, but you might also feel your facial muscles twitch slightly just around your eye. This twitch is just as brief as the thumb twitch and is a result of the TMS directly stimulating the facial nerves and muscles that run directly under your scalp. It is not painful. TMS can cause heating or movement of metallic objects in or near the head. In addition, the inactivation of pacemakers, medication pumps, cochlear prostheses and other implantable hardware may occur. Magnetic media such as credit cards, etc. and watches near the coil may also be damaged. To minimize this risk the researchers will have asked you about any metal implants which would exclude you from the study. There is a known risk of inducing a seizure during rTMS [56,57], however, it is not used in grant proposal.

Motor assessments: Motor assessments may occasionally cause fatigue; however you have the option to adjust your pace as needed to complete the test.

Protection to Human Subjects:

tDCS:
1) Strictly follow the inclusion and exclusion criterias
2) Study part 1 is a tolerabilty study. Adverse events are recorded carefully on every visit on every patients, if major response occurs, DSMB will be notified immediately.

MRI:
1) MRI screening form will be done at the time of recruitment, and patient will be excluded from study if he/she has contraindication for MRI or can not tolerate the MRI scanner
2) Headphone or earplug is provided to the study patient to minimize the noise from magnetic field.

TMS:

1) strictly follows the published guideline about TMS application. TMS is only applied by skilled and well trained staff in the center of brain stimulation.
2) rTMS will not be used in our grant application which has a risk of seizure.
3) Conduct a daily assessment after each study session to collect more information about side effect

Potential Benefit of the study:
There will be no other direct benefit to you from participating in this study except the one hour rehabilitation therapy; however, it is hoped that the information gained from the study will help in the treatment of future stroke patients with conditions like yours, and help the researchers to develop new rehabilitation treatment for stroke survivors in the future.

Data Safety Monitor Board and Data safety monitoring plan:

The DSMB will be composed of three of external members who are not involved with the study design or experiments: Dr. David Bachman(Chair of DSMB, MD, board certified neurologist, ex-IRB committee member), Christine Holmsted (DSMB member, DO and board certified Neurologist) and Andrea Boan (DSMB member, PhD biostatistician).

The DSMB member will meet every 6 months. If 2 or more major responses that occurs in the study part 1 at specfic current level, the study need to be stopped early. The DSMB will meet earlier than 6 months interval.

The chair will lead the meeting with two members, Specifically they will
1) protect the safety of the study participants;
2) review the research protocol, informed consent documents, amendments, and plans for data safety and monitoring;
3) evaluate the progress of the trial, including periodic assessments of data quality and timeliness, recruitment, accrual and retention, participant risk versus benefit, performance of the trial sites, and other factors that may potentially affect study outcome;
4) review study performance, make recommendations and assist in the resolution of problems reported by the Principal Investigator;
5) report to IRB on the safety and progress of the trial;
6) ensure the confidentiality of the study data and the results of monitoring
7) advise the IRB and the study investigators as to whether the protocol should continue as scheduled or undergo a modification due to a finding from the monitoring process

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