ORGANIZATION OF DETAILED PROTOCOL

Title: Effects of contralesional repetitive magnetic stimulation combined with fluoxetine on motor recovery in stroke patients
Protocol #:2014P001046
Date: 08/10/2017

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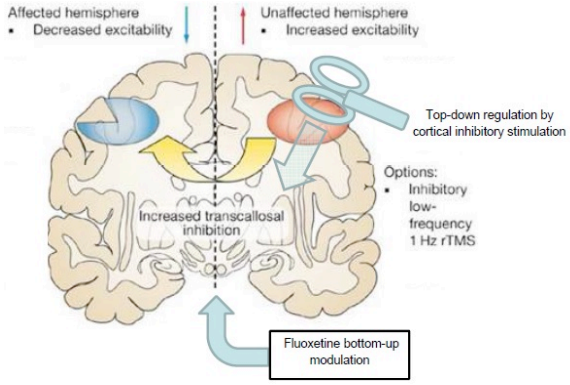

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Aim 1: Determine whether low-frequency rTMS of the unaffected M1 associated with fluoxetine offers an additional benefit on motor function over pharmacotherapy (fluoxetine) alone after three months of the combined therapy. *Our hypothesis is that low-frequency rTMS combined with fluoxetine will induce greater improvement in motor function as compared to sham rTMS and fluoxetine.*

Sub Aim 1.1*:* As to control for the effects of spontaneous recovery, we will also assess whether the superior effects of the combined effects will be superior to spontaneous recovery by comparing the results of the two groups against a placebo group (placebo fluoxetine and sham rTMS). *We hypothesize that both groups (combined treatment and fluoxetine alone) will have a superior effect as compared to placebo only*.

Aim 2: Determine whether fluoxetine combined with rTMS results in additional positive modulation of motor cortex excitability and cortical plasticity as compared to fluoxetine alone. *Our hypothesis is that active rTMS combined with fluoxetine will induce a greater increase in the amplitude of motor evoked potential and greater LTP-like (long-term potentiation) as indexed by PAS-25 of the affected hemisphere (M1) as compared to sham rTMS combined with fluoxetine.*

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V. SUBJECT SELECTION

We will recruit 45 patients with ischemic stroke. The desired sample size is calculated, with some assumptions, based on the amount of patients treated in Spaulding Rehabilitation Hospital and affiliate hospitals and clinics. The study eligibility criteria that will be used for this study are based on the FLAME trial (as we will investigate the additional benefits of combining fluoxetine with rTMS). We broadened the motor score criteria and time since stroke in order to significantly improve the impact of the study and improve our understanding of the mechanisms behind plasticity after stroke in a broad population.

Subjects will conform to the following criteria:

Inclusion Criteria:

- Ischemic infarction within the past 2 years post event that has caused hemiparesis or hemiplegia, as self-reported and/or confirmed by medical record.
- Older than 18 years old.
- Upper extremity weakness defined as a score of >11 and ≤56 on the arm motor Fugl-Mayer motor scale.
- Minimal pre-stroke disability defined as a score of <3 in the Modified Rankin Scale.
- Subjects need to be able to follow directions and participate in 2 hours of testing with short breaks.
- Subjects need to be able to provide informed consent.

Exclusion Criteria:

- Any substantial decrease in alertness, language reception, or attention that might interfere with understanding instruction for motor testing
- Excessive pain in any joint of the paretic extremity (not applicable to severe stroke subjects), as self reported
- Contraindications to single pulse TMS (will be used to measure cortical excitability) such as: history of seizures, unexplained loss of consciousness, any metal implants in the head, frequent or severe headaches or neck pain, any other electronic implanted medical devices such as pacemakers, defibrillators, or implant medication pump.

- Patients who are currently taking fluoxetine should accept a 5 week washout period before baseline (to be reconfirmed during baseline visit).
- Patients taking any other SSRI at the time of enrollment or in the previous month (the patients should accept a 5 week washout period before baseline - to be reconfirmed during baseline visit).
- Patients taking any other medication likely to have adverse interaction with SSRIs (all the medications the patient is taking will be carefully reviewed, as noted below in "Monitoring of important drug interactions").
- Active depression on admission to SRH defined by a score of 24 or higher in the Hamilton Depression Rating Scale (HAM-D)
- Concurrent medical condition likely to worsen patient's functional status in the next 6 months such as: cancer, terminal heart, kidney or liver disease, as self-reported and/or confirmed by medical record.
- Pregnancy.

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VII. STUDY OVERVIEW AND PROCEDURES

Study overview:
This study is a parallel, randomized, double blind, placebo controlled clinical trial. We will use the same duration and regimen of fluoxetine treatment as the FLAME trial as this trial showed that fluoxetine is efficacious when compared against placebo. Subjects will be randomized to one of three groups, where they will receive*: 1) fluoxetine + active rTMS, 2) fluoxetine + sham rTMS* or *3) sham rTMS + placebo fluoxetine*. With a total of 45 patients, 15 patients will be randomized to each group using a computer based randomization program.

This study is structured in 3 visits where baseline and post intervention assessments will be collected, 10 daily stimulation sessions (from 3rd until 12th visit), and subsequently, subjects will return for a weekly stimulation session for the next 8 weeks (visit 14th to 21th). Last visit will be the 22nd and the whole battery of assessments is going to be collected. A table of the study flow is presented below.

Protocol Details:
There will be ten stimulation visits (to be given over a period of 15 days), followed by weekly sessions of stimulation for the next 8 weeks. During each stimulation visit, rTMS equipment will be placed on the subject's unaffected hemisphere over the primary motor cortex (M1). They will receive either active or sham rTMS with fluoxetine – or sham rTMS and placebo. The 30 subjects randomized to receive sham rTMS will have the opportunity to enroll into an open label at the conclusion of their participation in the randomized portion of the trial. This will consist of 10 daily active stimulation sessions that can be carried out over the course of 2 weeks. Subjects will only receive rTMS and not the study drug, Fluoxetine.

Intervention Details:

Repetitive transcranial magnetic stimulation:

Low frequency rTMS stimulation (active stimulation)

- During this stimulation session, the participant will receive a session of low-frequency rTMS to the primary motor cortex of the unaffected cerebral hemisphere. The resting motor threshold (MT) of the first dorsal interosseous (FDI) in the affected and unaffected hemispheres will be measured. The participants will receive the rTMS over the area corresponding to the "hot spot" for stimulation as defined by motor threshold determination, as described by prior rTMS application studies (Rossini et al., 1994).

- Low frequency rTMS stimulation will be applied according to the following parameter: intensity of 100% MT, frequency of 1 Hz, 1200 pulses as a single, continuous train lasting 20 minutes. This intervention is associated with significant motor gains in chronic (Mansur, 2005)(Fregni, 2006) and acute stroke. In addition, it is a safer approach to be used in chronic stroke (Fregni review).

Sham rTMS stimulation:

- For sham rTMS stimulation, we will place the coil in the same location usually used for the active stimulation. We will also utilize the same stimulation parameters. However, we will replace the active coil with a sham coil to ensure no stimulation is actually provided.

Drug Intervention:

Fluoxetine:

- Subjects will receive fluoxetine 20 mg daily after enrollment during their baseline visit. They will take the study drug by mouth once daily from this day until the protocol is completed (for 90 days) - we will use the same regimen as in the FLAME study.
- The drug will be dispensed by the pharmacy and nursing staff of Spaulding while subjects are inpatient at the hospital. Upon discharge from Spaulding subjects will be given the remaining pills in their prescribed amount supply and instructed to take one each day until finished.
- If the subject is discharged to a skilled nursing facility a copy of the protocol and contact information will be sent with the patient and the medication. Study staff will call the facility to answer questions and enlist support in continuing the study medication there. Study drug compliance will be monitored weekly via self-report measures either during a study visit or by phone. Subject's responses will be kept in a compliance log.
- If the subject is enrolled as outpatient a licensed physician on the study staff will dispense the medication to the subject during the baseline visit.

Placebo control:

- Subjects will receive a placebo pill daily after enrollment during their baseline visit. They will take the study drug by mouth once daily from this day until the protocol is completed (for 90 days). The placebo will be dispensed by the pharmacy and nursing staff of Spaulding while subjects are inpatient at the hospital. Upon discharge from Spaulding subjects will be given the remaining pills in their prescribed amount supply and instructed to take one each day until finished. The procedure for taking the study drug and to monitor for compliance will be the same as for the patients receiving the active drug.

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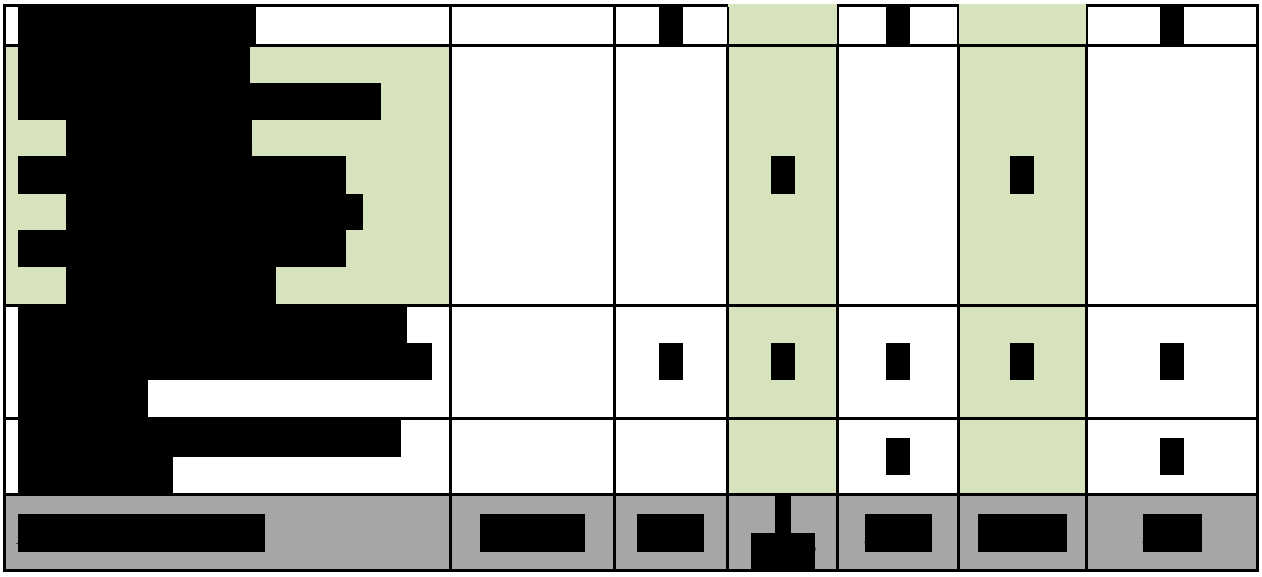

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Clinical Assessments:

A rater blind to the treatment arm will administer the following:

1. Jebson Taylor Hand Function Test (JTHF): This will be our primary outcome. This test was designed as a broad measure of hand function. It provides information on the time required for the subject to turn cards, pick up small objects, simulate feeding by picking up beans with a spoon, stack checkers, and lift empty and 500 g full cans (Jebsen et al., 1969). This instrument showed to be sensitive to measure motor changes induced by motor cortex stimulation (REVIEW marcel - BJ). When performing a certain sub-task, if the subject demonstrates the inability to complete that task and verbally acknowledges he/she cannot perform, we will move on to the next task and score appropriately.
2. Fugl-Meyer motor scale (FMMS): This instrument was the main outcome used in the FLAME study and is widely used for assessment of motor recovery after stroke. (Gladstone et al, 2002).
3. Modified Ashworth Scale: This instrument is a 6-point rating scale that is used to measure muscle tone. This test is performed by moving the body part through the joint range of motion (ROM), with no specification as to the speed of the movement.
4. Beck Depression Inventory (BDI): This 21-item multiple-choice test measures the presence of and the degree of depression in adults.
5. Mini Mental State Examination (MMSE): The MMSE is a brief screening instrument used to assess cognitive abilities. We will be using this assessment as a baseline evaluation and a follow- up. Consistency of MMSE scores should suggest that a subject had no cognitive changes throughout the intervention period that may have affected test performance or carryover of the program.
6. National Institute of Health Stroke Scale (NIH SS): is a tool used to objectively quantify the impairment caused by a stroke. It is composed of 11 items, each of which scores a specific ability between a 0 and 4.
7. Visual analogue scale (VAS) for anxiety: This tool is a visual scale of 0-10 where the subject can rate their level of anxiety where 0 is no anxiety, and 10 is the worst anxiety that the subject has ever felt.
8. Visual Analogue Scale (VAS) for pain /comfort: This tool is a visual scale of 0-10 where the subject can rate their level of pain where 0 is no pain, and 10 is the worst pain that the subject has ever felt.
9. Side Effects / Adverse event tracking Questionnaire for rTMS: After each session, subjects will complete a questionnaire to evaluate potential common adverse effects rTMS (headache, neck pain, itching and redness at the site of stimulation) on a 5-point scale.
10. The Antidepresant Side-Effect Checklist (ASEC): The ASEC is a questionnaire that assesses the possible appearance of side effects related to the use of common antidepresants, their severity and if they are linked or not to the drug (Uher, 2010).
11. Blinding Questionnaire: to be performed at the end of the daily stimulation sessions and at the end of the weekly stimulation sessions.. This questionnaire will ask the rater and the subjects whether the intervention was sham or active rTMS . The confidence of these responses will be rated from 0 to 5 – 0 being no confidence and 5- total confidence. If the subject is interested in knowing what stimulation he/she received, the co-investigator may inform the subject when his/her participation in the trial has ended. .

12. A log of the hours of therapy that patients are receiving and the meditations they are taking will be kept and updated every week.

Assessment of cortical excitability and plasticity – single and paired pulse TMS and PAS:

Aim 1: Determine whether low-frequency rTMS of the unaffected M1 associated with fluoxetine offers an additional benefit on motor function over pharmacotherapy.

We will investigate changes in cortical excitability by evaluating the motor evoked potential (MEP) and the resting motor threshold (MT) (with the same methods as in our previous study (Fregni et al., 2006b)). We will also measure intracortical excitability using the technique of paired-pulse, and interhemispheric differences using transcallosal inhibition. Both the affected and unaffected M1 will be studied.

We will investigate the resting motor threshold (measured following the technique described by (Rossini et al., 1994)) before and after stimulation. For the MEP study, we will initially adjust TMS intensity to achieve a baseline MEP in the first dorsal interosseous of about 1 mV peak-to-peak amplitude before intervention. Stimulation intensity will be kept constant for each subject throughout the experiment. The MEPs will be recorded and stored in a PC computer for off-line analysis. We will record 10 MEPs for each time point (immediately before and after the rTMS stimulation) and average their peak-to-peak amplitude and area-under-the-curve.

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For the first aim, the primary outcome will be motor function changes as indexed by Jebsen Taylor Hand Function test and secondary outcome will be Fugl-Meyer motor scores. Differences between the two groups will be tested using Student's t-test and, in addition, we will adjust for important baseline variables and also test the time effect in general mixed longitudinal models. Secondary analyses will be conducted in an exploratory manner (no correction for multiple comparisons). [We will also conduct exploratory correlational analysis as to understand whether beneficial effects of the combined intervention (active rTMS and fluoxetine) are associated with any baseline characteristics as to explore potential predictors of the effects of treatment. This result may also be used along with results for aim 2 to understand the mechanisms of the combined treatment.]

[For subaim 1: the primary outcome will be motor function changes as indexed by Jebsen Taylor Hand Function test and secondary outcome will be Fugl-Meyer motor scores. We will conduct the same analysis as aim 1 (Differences between the two groups will be tested using Student's t-test with adjustment for covariates) but in this case we conduct two comparisons (real rTMS+fluoxetine vs. sham rTMS+placebo fluoxetine and sham rTMS+fluoxetine vs. sham rtMS+placebo fluoxetine)]

For the second aim, we will initially build a mixed ANOVA model in which the dependent variable will be the parameters of cortical excitability and plasticity (PAS) indexed by

neuropsychological measures. The independent variables will be group [(active and placebo control)], time and the interaction group/time. [If we find a significant interaction effect between group and time, we will confirm our hypothesis that TMS induces a significant differential plasticity when compared to fluoxetine alone. Furthermore we will conduct additional analysis in which we will change the groups in the variable group (real rTMS+fluoxetine vs. sham rTMS+placebo fluoxetine and sham rTMS+fluoxetine vs. sham rtMS+placebo fluoxetine)

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