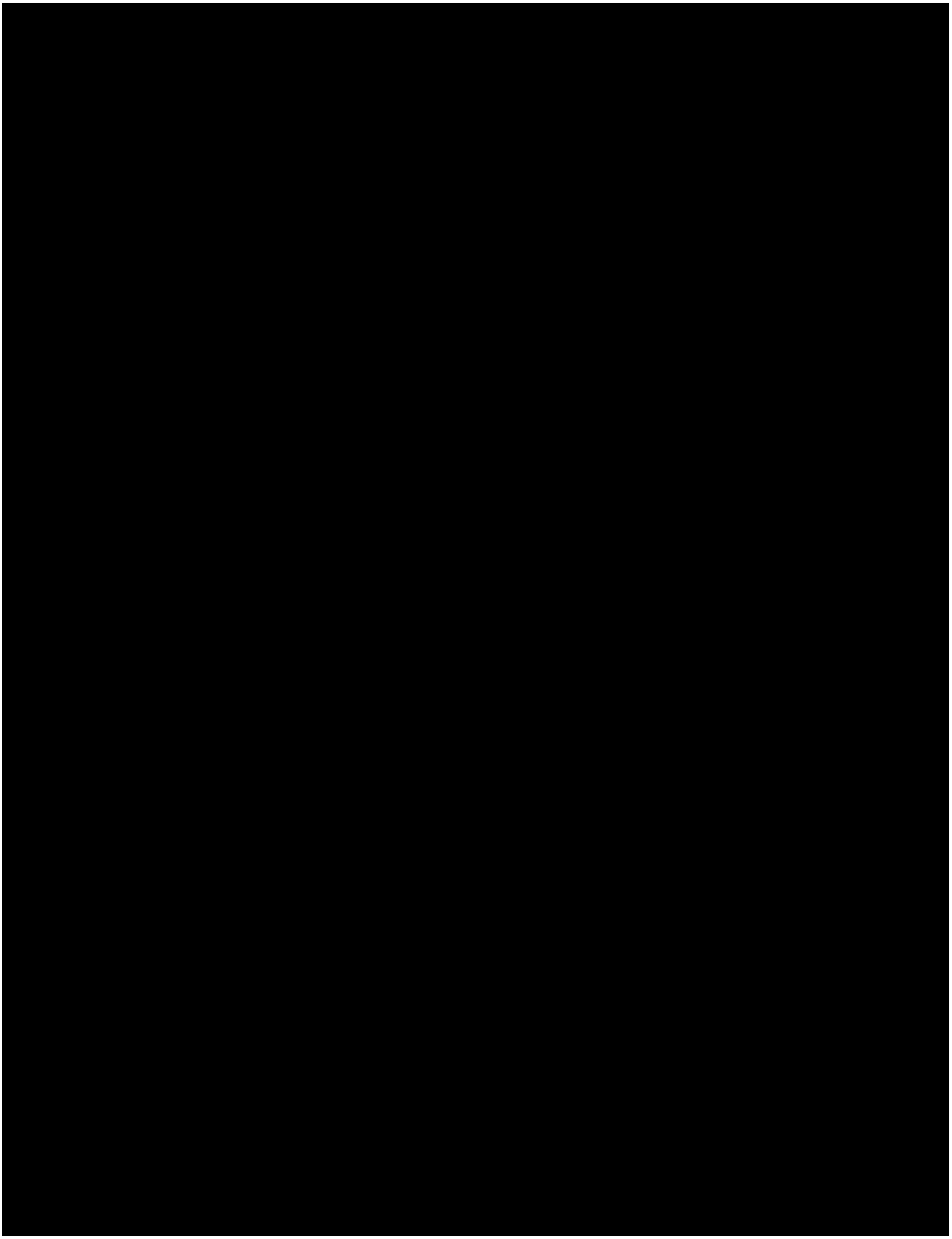
ORGANIZATION OF DETAILED PROTOCOL

Title: Optimizing Rehabilitation for Phantom Limb Pain Using Mirror Therapy and transcranial Direct Current Stimulation (tDCS).

Protocol # 2015P001065

Date: 08/14/2019



Sponsors

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II. SPECIFIC AIMS

The specific aims of this experiment are as follows:

- **<u>Aim 1: To evaluate the efficacy of tDCS and mirror therapy (MT) as rehabilitative tools for the management of chronic PLP patients.</u>**
  We will explore the aim 1 in a randomized factorial controlled trial in which patients will be assigned to one of four groups: active tDCS and active MT; sham tDCS and active MT; active tDCS and sham MT (which consists of using a covered mirror for the therapy); and both sham tDCS and sham MT (covered mirror).

The primary end point will be severity of pain measured by changes in PLP as indexed by a Visual Analog Scale (VAS). The study will require a total of 13 weeks to be completed. The first week, each patient will give daily reports of their pain using a VAS, accounting for their PLP experience in the past 24hs. This initial baseline week will be followed by 4 weeks of treatment (tDCS and/or MT) and a final 8-week follow-up period without any treatment to examine long-lasting effects of each therapy. *Our hypothesis is that the combined intervention (tDCS and MT) will result in a greater pain reduction as compared with any therapy alone and no therapy*.

- **Aim 2: To examine the mechanisms underlying PLP using two neurophysiological techniques.**
  For each of the four groups described in aim 1, we will determine the neural correlates of the effect. We will use single-pulse and paired-pulse transcranial magnetic stimulation (TMS) to assess cortical mapping and cortical excitability changes associated with cortical reorganization. We will also use functional magnetic resonance imaging (fMRI) to assess brain changes, including the quantification of maladaptive cortical reorganization. Using fMRI, we will determine the changes in Blood-Oxygen-Level dependent activation between the sensory-motor cortex of the affected and unaffected side after the treatment. We will compare these neurophysiological parameters before and after treatment in each of the four groups. *Our hypothesis is that the combined group (tDCS and mirror therapy) will have a greater activation than any therapy alone and also than the no therapy group (sham tDCS and covered mirror). This effect will also be correlated with pain reduction*.

IV. SUBJECT SELECTION

In this study, we will recruit 132 subjects with phantom limb pain (PLP) of traumatic etiology (congenital or diabetic amputees will be excluded since these patients may have a different neuroplastic profile). The subjects will be recruited by 2 main neurorehabilitation study centers, one at neuromodulation center - Spaulding rehabilitation Hospital and one at University of Sao

Paulo Brazil (IMREA). Subjects will need to meet all of the following inclusion criteria and none of the following exclusion criteria:

Inclusion Criteria:

1. Able to provide informed consent to participate in the study.
2. Subject is older than 18 years.
3. Unilateral lower limb amputation.
4. 3 months of phantom limb pain (experienced regularly for at least once a week) after the amputated limb has completely healed.*
5 Average pain of at least 4 on a numeric rating scale in the previous week (NRS; ranging from 0 to 10).
6 If the subject is taking any medications, dosages must be stable for at least 2 weeks prior to the enrollment of the study.

*The healing status of the limb will need to be confirmed by the subject's physician or by the physician of the protocol Dr. David Crandell (Co-Investigator).

Exclusion Criteria:

1. Pregnancy or trying to become pregnant in the next 2 months.
2. History of alcohol or drug abuse within the past 6 months as self-reported.
3. Presence of the following contraindication to transcranial direct current stimulation and transcranial magnetic stimulation
   - Ferromagnetic metal in the head (e.g., plates or pins, bullets, shrapnel)
   - Implanted neck or head electronic medical devices (e.g., cochlear implants, vagus nerve stimulator)
4. Head injury resulting in permanent neurological deficits, such as cognitive or motor deficits, as self-reported.*
5. Unstable medical conditions (e.g. uncontrolled diabetes, uncompensated cardiac issues, heart failure or chronic obstructive pulmonary disease)
6. Uncontrolled epilepsy or prior seizures within the last 1 year
7. History of unexplained fainting spells or loss of consciousness as self-reported during the last 2 years.
8. History of neurosurgery, as self-reported.
9. Mirror Therapy within 3 months prior to enrollment

* permanent neurological deficit that may interfere with the assessments.

The safety of tDCS in pregnant population (and children) has not been assessed and therefore pregnant women (and children) will be excluded. Women of child-bearing potential will be required to take a urine pregnancy test during the screening process.

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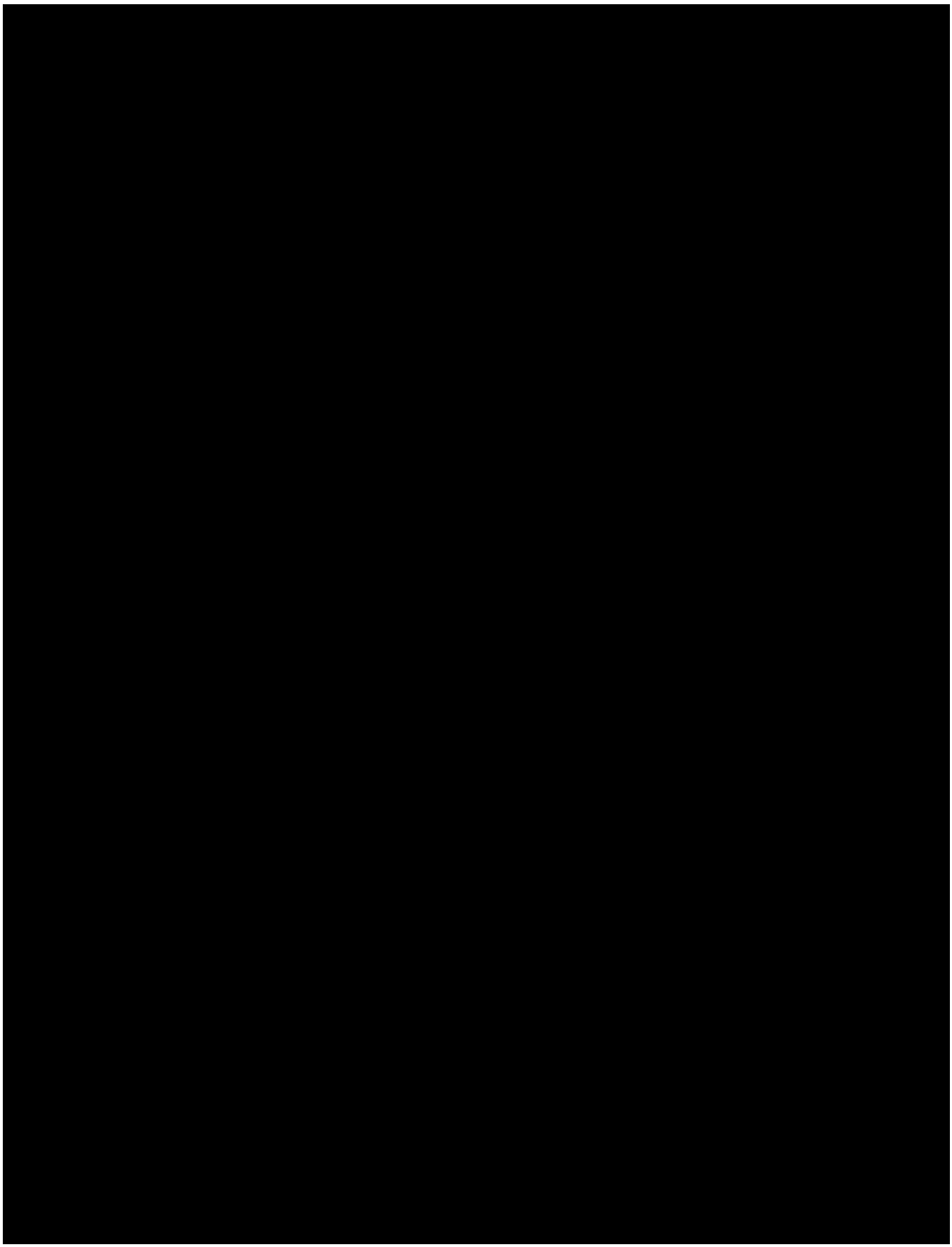
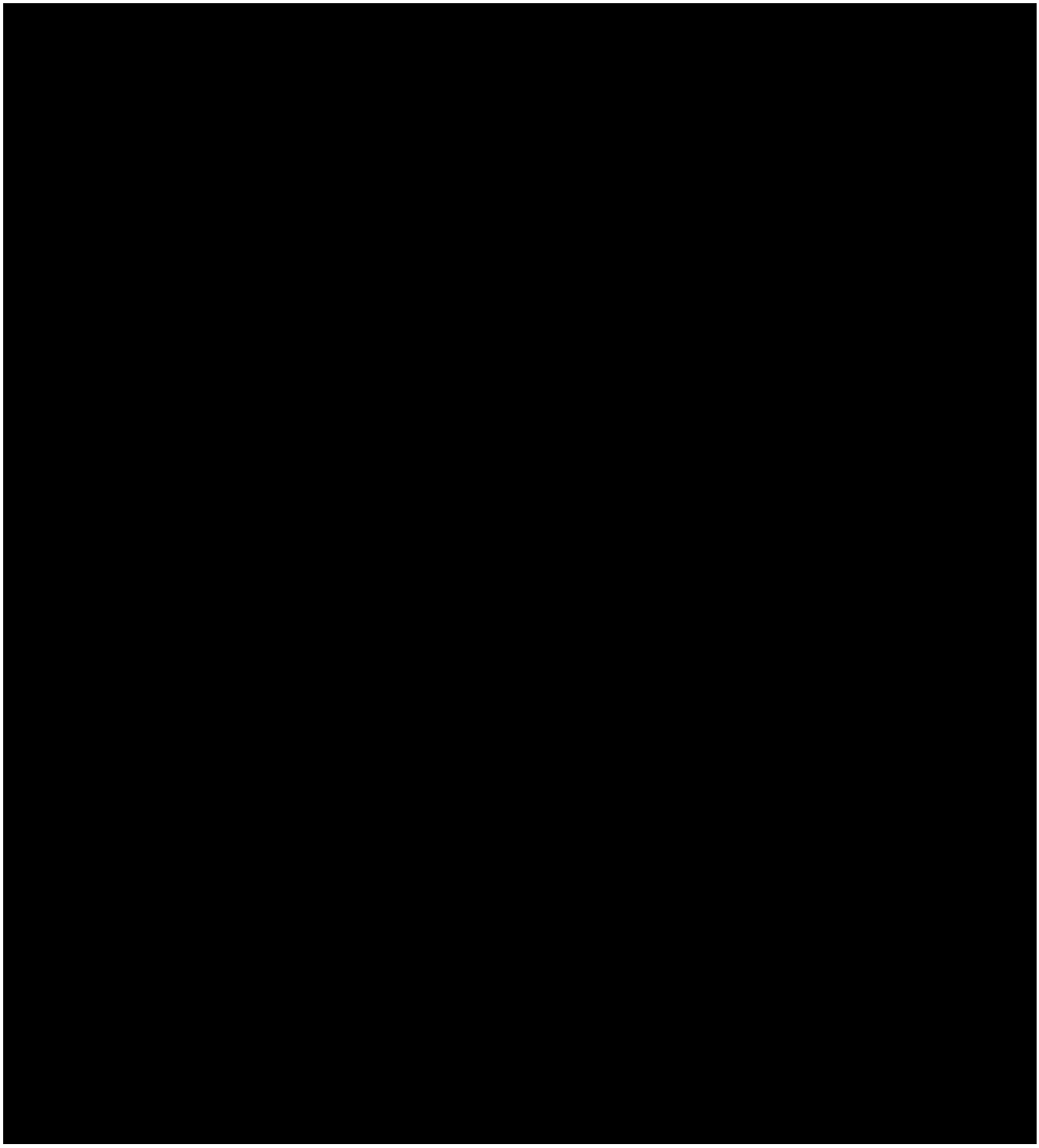




V. STUDY PROCEDURES

We will recruit 132 participants with major lower unilateral limb amputation and phantom limb pain in the chronic phase.

The patients will be assigned to one of four groups: the first will receive active tDCS and active MT; the second will receive sham tDCS and active MT; the third will receive active tDCS and sham MT; and the fourth will receive both sham tDCS and sham MT. Subjects and outcome evaluators will be blinded to the intervention assigned. The study will be done for a total of 13 weeks. The study procedures will be done in the same way in both study centers. Data collection will be performed according to the IRB approved protocol by trained and capable research staff. The Neuromodulation Center (Spaulding Rehabilitation Hospital) will be responsible for providing training and responsible for monitoring of the study, including maintaining regular contact with the University of Sao Paulo site through telephone contact. This phone contact will be logged and stored at the Regulatory binder. The data will be collected by staff members (PI, co-investigators and study staff) familiar with the protocol and the tDCS and TMS technology. The care, safety, and comfort of the subjects will be under the supervision of a physician and therapist at each trial site. The local IRB at each respective institution will be responsible for overviewing the conduct of the study trial locally. Both IRB institutions approved the research protocol. Unidentifiable data from the local institution in Brazil will be shared with the main site (Boston) via IRB approved data base RedCap. There will be no sharing of PHI between the sites.

The subjects randomized to receive sham tDCS and/or sham MT will have the opportunity to enroll into an open label portion of the study at the conclusion of their participation in the randomized portion of the trial, including 10 sessions of tDCS combined with MT and additional questionnaire data.

Study Outline

Pre-screening Procedures:
During the pre-screening process, the subject will contact a co-investigator usually via a phone call. During this call, the co-investigator will discuss in greater depth the details of the study, explain the study procedures and encourage the subject to ask questions. In the privacy of the laboratory, the co-investigator will ask the subject questions from the following:

1) Phone screening questionnaire

Once this information is collected, the co-investigator will consult with the PI regarding the eligibility of the subject, who will then give approval for the subject to come to our laboratory for the screening procedure.

Visit 1
Screening Visit – (Approximate Time: 1 hour)

Screening Procedures:
At Screening the PI and/or a co-investigator will conduct, once more a review of inclusion/exclusion criteria to determine the subject's eligibility for enrollment. Study procedures will be reviewed with the subject, and documentation of informed consent will be obtained.

At Screening the following procedures will be completed:

- Discuss study-specific procedures with the subject.

- Review inclusion and exclusion criteria.
- Obtain a signed and dated consent form.
- Conduct a Demographics Survey, Brief Medical History and Beck depression inventory.
- fMRI screening questionnaire*.
- Urine pregnancy exam (if applicable).

* If the subject is not eligible for an fMRI at the time of the study we will not perform this assessment as fMRI is not required for study eligibility and it is only used as a secondary outcome.

Randomization:
Once eligibility and consent have been approved and completed, randomization will occur using the randomized list generated by an automatic web-base randomization program (www.randomization.com). Subjects will be randomly assigned to one of the four groups in a 1:1:1:1 allocation ratio. We will use stratified randomization methods with random block sizes of blocks of 4 and 8. Patients will be stratified based on their baseline pain levels (two strata: less or equal of average 6 in VAS or more than 6 in VAS) and by study center (IMREA or SRH-Neuromodulation Lab). Randomization order will be kept in sealed envelopes; therefore subjects will get their assignment according to the order of entrance in the study (for instance, subject 1 will be assigned the first envelope that will contain his/her assignment according to this block randomization list).

Visit 2
Baseline Visit – (Approximate Time: 2 hours)
This visit might be completed on the same day as the screening visit if time allows and the subject agrees.

Baseline assessments:

- Visual Analogue Scale (VAS) for Pain
- Visual Analogue Scale (VAS) for Stump pain
- Visual Analog Scale (VAS) for Phantom Limb Sensation
- Visual Analog Scale (VAS) for Phantom Limb Telescoping
- Adapted Groningen Questionnaire After Arm Amputation
- Pain and Medication use diary*
- Beck Anxiety Inventory
- Mini Mental State examination (MMSE)
- Stroop Test
- Health Survey SF-36
- Single and paired pulse TMS
- Side Effects Questionnaire for TMS

Visit 3
fMRI Evaluation – (Approximate Time: 1 hour)

***Visits 1-3 may be completed on the same day or broken up into two or three separate visit as the subject prefers*

Visit 4 – 12 (Approx Time: 45 minutes)
Intervention visits: tDCS + Mirror Therapy
This visit will be scheduled within 1 week after the baseline measurements are done.

In the first 2 weeks subjects will receive tDCS (*sham or active) and mirror therapy (sham or active). There will be 10 stimulation visits to be completed in these 2 weeks (ideally Monday to Friday, 5 days each week). The subject will receive 20 minutes either of active or sham tDCS per day and 15 min of active or sham mirror therapy sessions. The subjects will receive the intervention according to the prior randomized group.

Before stimulation, the subject will complete the following assessments:

- VAS Phantom Limb Pain
- VAS for Stump pain
- VAS for Phantom Limb Sensation
- VAS for Phantom Limb Telescoping

After each session, subjects will complete the following assessments:

- Side Effects Questionnaire for tDCS
- VAS Phantom Limb Pain
- VAS for Stump pain
- VAS for Phantom Limb Sensation
- VAS for Phantom Limb Telescoping

Visit 13
Last day of tDCS session + Mirror Therapy – (Approx Time: 2 hours)

Before stimulation, the subject will complete the following assessments:

- VAS Phantom Limb Pain
- VAS for Stump pain
- VAS for Phantom Limb Sensation
- VAS for Phantom Limb Telescoping

After each session, subjects will complete the following assessments:

- Visual Analogue Scale (VAS) for phantom limb pain
- Visual Analogue Scale (VAS) for Stump pain
- Visual Analog Scale (VAS) for Phantom Limb Sensation
- Visual Analog Scale (VAS) for Phantom Limb Telescoping
- Pain and medication use diary
- Stroop Test
- Beck Anxiety Inventory

- Beck Depression Inventory
- Side Effects Questionnaire for tDCS
- Blinding Questionnaire
- Single and paired pulse TMS
- Side Effects Questionnaire for TMS

After the first 2 Weeks combined intervention (sham/active tDCS plus sham/real mirror therapy) at the Neuromodulation Center in front of the therapist, subjects will continue mirror therapy for 10 additional sessions over the course of ~ 2 weeks at home.

2 weeks of home Mirror Therapy – (Approx Time: 15 min)

During this 2 week period the co-investigator will try to contact the subjects 3 times per week to assess adherence to the MT treatment and acquire pain diary and medication changes data. We are going to try maximum 2 attempts per day.

Visit 14:
Last day of mirror therapy - (Approx Time: 3 hours)
This visit will be scheduled in the last MT home session and the following assessments will be performed. This visit can be divided in two days if time allows and the subject agrees.

Subject can perform the last mirror therapy session either at home or at the Neuromodulation Center.

After the MT session, subject will complete the following assessments:
- Visual Analogue Scale (VAS) for phantom limb pain
- Visual Analogue Scale (VAS) for Stump pain
- Visual Analog Scale (VAS) for Phantom Limb Sensation
- Visual Analog Scale (VAS) for Phantom Limb Telescoping
- Pain and Medication uses diary
- Stroop Test
- Health Survey SF-36
- Patients' Global Impression of Change (PGIC) scale
- Beck Anxiety Inventory
- Beck Depression Inventory
- Single and paired pulse TMS
- Side Effects Questionnaire for TMS
- fMRI evaluation

Visit 15: fMRI Evaluation – (Approximate Time: 1 hour)

***Visits 14 & 15 may be completed on the same day or broken up into two separate visit as the subject prefers*

Visit 16:
First Follow Up Visit – (Approx Time: 1 hour)
This visit will be scheduled ~ four weeks (+/- 5 days) after the last MT home session and the following assessments will be performed:

- Visual Analogue Scale (VAS) for phantom limb pain
- Visual Analogue Scale (VAS) for Stump pain.
- Visual Analog Scale (VAS) for Phantom Limb Sensation
- Visual Analog Scale (VAS) for Phantom Limb Telescoping
- Beck Anxiety Inventory
- Beck Depression Inventory
- Pain Medication uses diary

***Visit 16 may be completed by terms of phone interview as the subject prefers.*

Visit 17:
Last Follow Up Visit - (Approx Time: 2 hours)
This visit will be schedule ~eight weeks (+/- 5 days) after the last MT home session and the following assessments will be performed:

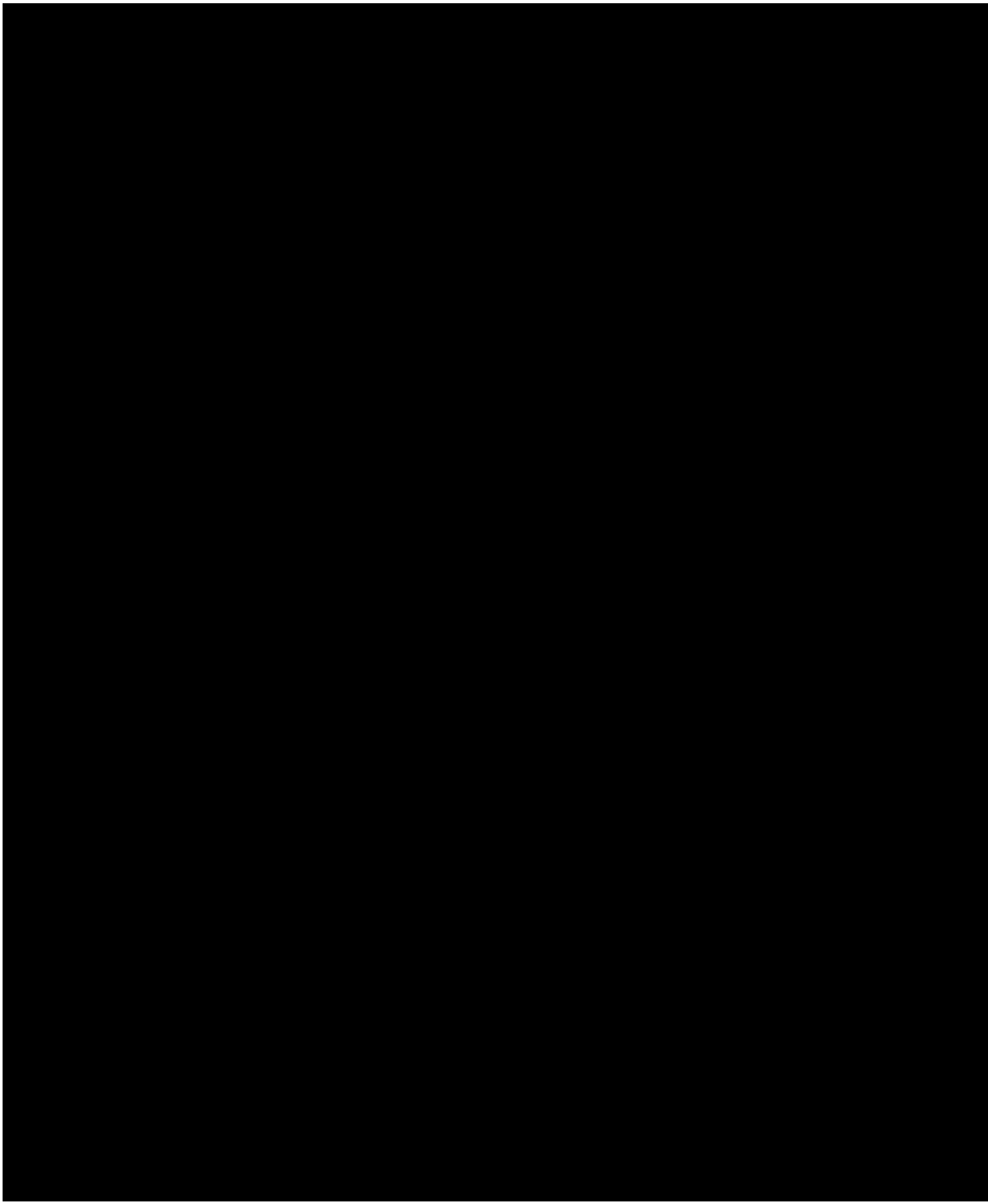
- Visual Analogue Scale (VAS) for phantom limb pain
- Visual Analogue Scale (VAS) for Stump pain
- Visual Analog Scale (VAS) for Phantom Limb Sensation
- Visual Analog Scale (VAS) for Phantom Limb Telescoping
- Pain Medication uses diary.
- Stroop Test.
- Health Survey SF-36.
- Beck Anxiety Inventory
- Beck Depression Inventory
- Single and paired pulse TMS
- Side Effects Questionnaire for TMS
- Blinding questionnaire

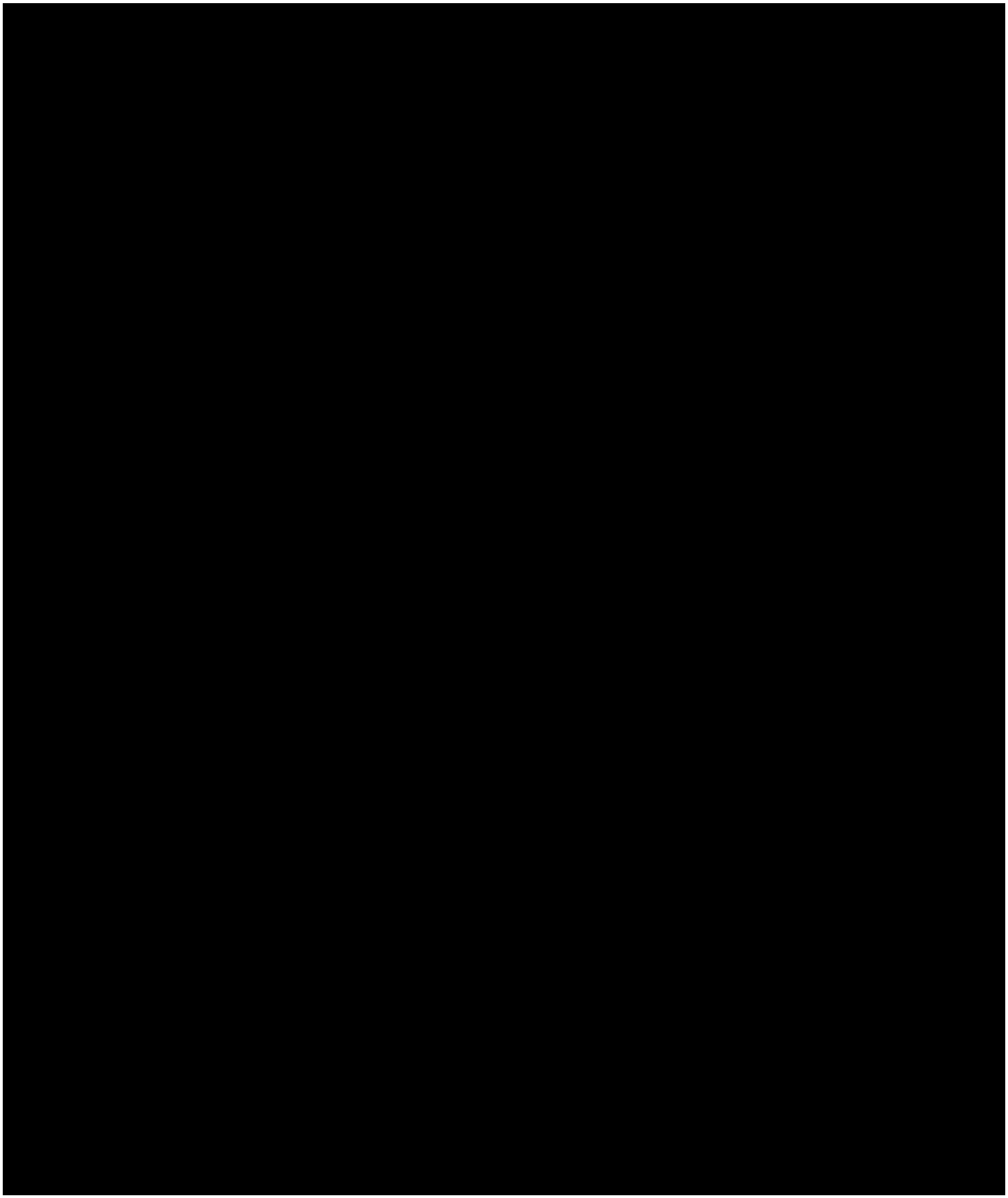
**After visit 14 until the end of the trial participation, the co-investigator will try to contact the subjects 3 times per week to acquire pain diary and medication changes data. We are going to try maximum 2 attempts per day.

The schedule for the subjects can be seen in table 1, which illustrates the procedures that will be performed at each visit:

Table 1: Visits Schedule

	Consent and Screening	**Basel ine**	**fMRI Visit**	**Interventions Session**	**fMRI Visit**	**Follow-Up**





observed in the mirror to their phantom limb and to keep their attention focused on the task. Instructions will be explained verbally, demonstrated by a therapist, and performed by the subject in front of the therapist during the first two weeks (the MT sessions will be scheduled back-to-back with the tDCS sessions). After this training, subjects will continue MT everyday for 2 more weeks at home. Subjects will be instructed to stop MT if it intensifies their pain, and to document if this happens

- Sham Mirror Therapy (Covered MT): Subjects will be asked to perform movements in the same way that the active group but with a covered mirror.

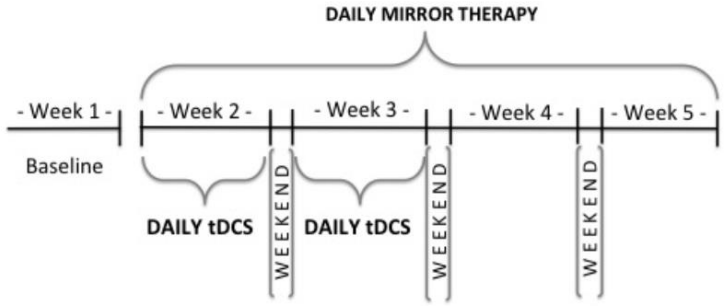


Figure 1: Schematic view of the experiment in time points: baseline, procedures, subjects enrolled intervention, first follow-up and second follow up.

DESCRIPTION OF ASSESSMENTS:
A rater blind to the treatment arm will administer the following tests:

Visual Analogue Scale (VAS) for Pain: The VAS is a common assessment used which asks subjects to self-reportedly measure their pain on a visual scale (i.e., unbearable to none). We will use a VAS to determine subjects' pain scores. Subjects will rate their pain from 0 – indicating no pain at all, to 10 – indicating the worst pain felt. This scale is also colored, from green (at 0) to red (at 10), as a visual indicator of pain. This assessment tool is frequently used in many research studies evaluating pain levels [15, 27, 34, 35].

Visual Analogue Scale (VAS) for Stump Pain: any painful sensation in the stump. Subjects will rate their stump pain from 0 – indicating no pain at all, to 10 – indicating the worst pain felt. The scale will include colors to help in identifying the correct response [15].

Visual Analog Scale (VAS) for Phantom Limb Sensation: all non-painful sensations in the amputated part of the limb. Subjects will be presented with a scale starting at 0- No phantom limb sensation, to 10 – Full sensation of the amputated limb. The scale will include colors to help in identifying the correct response [15].

Visual Analog Scale (VAS) for Phantom Limb telescoping: refers to the shrinking and retraction of the phantom towards the residual limb. Subjects will be presented with a scale starting at 0 - indicated that the phantom was enlarged, and 10 meant that the phantom was completely retracted into the stump the scale will include colors to help in identifying the correct response [15].

Adapted Groningen Questionnaire after Arm Amputation: This questionnaire is originally meant to obtain information's concerning complaints that may be developed after arm amputation. We adapted the current arm version for lower limb amputation. This questionnaire [36] has been used in several clinical trials assessing PLP.

Pain and medication diary: To help monitor pain levels and medication use information, as well as safety. Subjects will be asked to record the number of phantom limb episodes on a daily basis, using a pain diary. They will record the intensity of the strongest episode as well as phantom limb sensation and stump pain on a colored visual analog scale included in the diary, where 0 represents no pain at all and 10 represents the highest pain the patient has ever felt. Moreover subjects will record their current medications and dosages daily in a pain medication diary, until completion of the study.

Beck Depression Inventory: This self-report inventory consists of 21 multiple-choice questions and is widely used method to classify depression severity. It assesses for the presence of several symptoms related to depression, such as irritability, hopelessness and decreased cognitive performance. Physical symptoms such as weight loss and fatigue are also included. This instrument has been used previously to evaluate depression severity in patients with phantom limb pain [37], as well as in other chronic pain conditions [31, 38, 39]. If the subject appears at-suicidal risk (detected by the BDI questionnaire- as defined by a score of ≥ 1 on question 9), the medical coverage will be contacted immediately by research staff to provide further evaluation and action to protect the subject (i.e. if the subject needs to be escorted or referred to the MGH ED Acute Psychiatry Service). In addition, if a subject has moderate to severe depression (defined by a score of ≥ 20 in the Beck Depression Inventory), the investigator will strongly recommend the subject search for depression management centers and encourage them to speak to their primary care physician). If the BDI questionnaire is performed over the phone (visit 16) the same procedure described above will be performed over the phone by the research staff (the research staff will discuss with the medical coverage and follow his/her plan of action according to above while subject is on the phone).

Beck Anxiety Inventory: This self-report inventory consists of 21 multiple-choice questions about how the subject has been feeling in the last week, expressed as common symptoms of anxiety (such as numbness and tingling, sweating not due to heat, and fear of the worst happening). It is designed for an age range of 17–80 years old. Each question has the same set of four possible answer choices, which are arranged in columns and are answered by marking the appropriate one with a cross [40].

Mini Mental State examination (MMSE): This is a sensitive, valid and reliable 30-point questionnaire that is used extensively in clinical and research settings to measure cognitive impairment. This instrument will be used as a brief screening of cognitive abilities. It will be used as a baseline evaluation [41].

Quality of Life Assessment (Short version of SF-36): The short version of the SF-36 health survey is used as a measurement of quality of life. It provides a profile of functional health and well-being scores. It is also used as a psychometrical index of physical and mental health. This

instrument is widely used as a quality of life assessment in patients after an amputation and those suffering from phantom limb pain [42-45].

Stroop test: In this task the subject is presented with names of colors written in the same color or in a different color, thus on the one hand the word names a color (red) and is written in another color (blue). In this task, the automatized behavior (reading) is in conflict with the desired response (naming the color). The Subject has to inhibit/suppress the automatic response of reading and naming the color the word is written in. The Stroop is one of the most commonly used tools for determining attentional problems, also used to assess executive function and working memory [46, 47]. Stroop test will be applied as a preliminary safety assessment of cognitive changes from baseline to post and follow-up visits.

Side Effects Questionnaire for tDCS: At each stimulation session, subjects will complete a questionnaire to evaluate potential adverse effects of tDCS (tingling, burning sensation, headache, neck pain, mood alterations) and mirror therapy (anxiety, grief, dizziness) on a 4-point scale (None, mild, moderate and severe). The subjects will be asked whether they have experienced any side effects in an open-ended manner and they will then be specifically asked about headache, neck pain, scalp pain, scalp burns, tingling, skin redness, sleepiness, trouble concentrating, and acute mood change. If any side effects are reported, the degree of relatedness to the intervention will be assessed on a 5-point scale. This type of adverse events questionnaire has been used frequently in our previous tDCS studies [31], including in patients with phantom limb pain [48].

Side Effects Questionnaire for TMS: At each TMS assessment session, subjects will complete a questionnaire to evaluate potential adverse effects of rTMS (headache, neck pain, itching and redness at the site of stimulation) on a 5-point scale [49].

tDCS blinding questionnaire: after the stimulation session, subjects will complete a questionnaire to determine if our blinding methods were effective. We are using a 30s sham montage, just as we use in our other trials, keeping the device on the subject for the duration of the session [50].

Patient's Global Impression of Change scale: subjects will complete a questionnaire to assess their perception of change (if any) in the activity limitations, symptoms, emotions and overall quality of life after their participation in intervention's visits of the trial [51].

Single and Paired Pulse TMS: Subjects will undergo several sessions of TMS to assess cortical excitability and cortical reorganization. For TMS assessment we will use Magstim BiStim² TMS device (Magstim ®). We will study the most distal muscle (i.e. biceps or quadriceps) of the amputated limb. In addition, if MEP is not obtained in the leg, the MEP will be assessed in the first dorsal interosseous. We will initially investigate changes in cortical excitability evaluating the motor evoked potential (MEP) and the resting motor threshold (MT); we will use the same methods as in our previous study [52], as well as short-interval intracortical inhibition (SICI) and intracortical facilitation (ICF) using the paired-pulse technique. We will investigate both contralateral and ipsilateral primary motor cortex (in relation to the amputated limb). We will investigate the resting motor threshold (MT), measured following the technique as described by

Rossini et al., where resting MT will be definssed as the lowest stimulus intensity to evoke a MEP of 50 μV in five of 10 trials in the relaxed muscle [53]. We will record this in both primary motor cortices when possible. For the MEP study, we will initially adjust TMS intensity to achieve a baseline MEP in the selected muscle of about 1 mV peak-to-peak amplitude before intervention. Stimulation intensity will be kept constant for each subject throughout the evaluation sessions. The MEPs will be recorded and stored in a computer for off-line analysis. We will record 10 MEPs for each time point (immediately before and after the tDCS treatment for both motor cortices) and average their peak-to-peak amplitude and area-under the-curve. The short-interval intracortical inhibition (SICI) and intracortical facilitation (ICF) will be measured using the paired-pulse technique [54], in both primary motor cortices. A suprathreshold test stimulus adjusted to an MEP amplitude of 1 mV will be preceded by a subthreshold conditioning stimulus (CS; 80% MT) at interstimulus intervals of 2, 3, 6, 9, 10, and 12 ms, which samples inhibitory (2, 3ms, SICI) and excitatory (9, 10, 12 ms, ICF) windows, respectively. Ten stimuli will be applied at each interval in a randomized order. The percentage of inhibition or facilitation for each ISI before and after treatment will be calculated. Cortical mapping: eight stimulations at 120 % stimulus of rMT intensity (posterior to anterior current) will be delivered to each of fifteen sites forming a 3 x 5 grid, with a constant 1.5 cm distance between sites, over the primary motor cortex (M1). At each stimulation site, the peak-to-peak amplitudes of the recorded motor evoked potentials (MEPs) will be measured and averaged offline.
The map CoG will be computed for the medio-lateral (x) coordinates using the formula: $CoGx = (\Sigma xi * MEPi) / \Sigma MEPi$ where MEPi represents the mean amplitude of the MEPs produced at one site. The MV will be calculated as the sum of the average MEP amplitude at each active site, where an active site was defined as a site at which the mean MEP amplitude was at least 0.05mV.

Functional magnetic resonance imaging (fMRI): to quantify maladaptive cortical reorganization before and after treatment. Each fMRI exam will be performed at the Boston University Medical Center, Center of Biomedical Imaging; this is a fee-for-service, and they are not engaged in the research. The BU Medical Center has an extensive and expanding inventory of state-of-the-art imaging facilities. One of the core technologies being developed and used at the center are Magnetic Resonance Imaging (MRI) and Spectroscopy (MRS). Additionally for this visit an fMRI survey (BU Safety Screening Form)form will be filled out by the radiation technician or study investigator (NINDS CDE) [26].
We decided to conduct a longitudinal design sessions (baseline and post-treatment). To minimize the limitations of this longitudinal analysis we will use an algorithm of initialization in the FreeSurfer processing stream [55].

The fMRI sessions will be conducted using the following stimuli and task detailed below and in Figure 2.

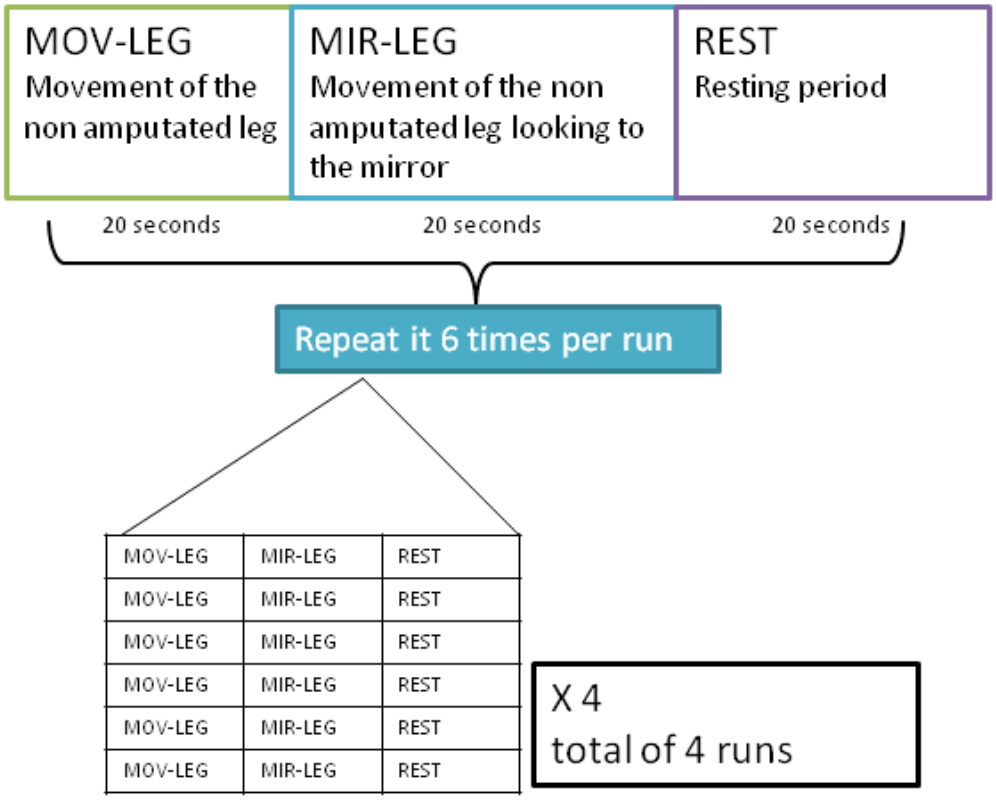


Figure 2. The scheme shows tasks performed during fMRI sessions.

We based this design in an experiment investigating activation of motor networks associated with observation and imagination of the lower limbs movements [56]. The stimuli will consist of the movement of the lower limb (non amputated limb), followed by the same movement but now the participant will be able to observe his/her leg moving and the leg image in the mirror through a online video.

The following three conditions will be therefore investigated:

1. Movement of the leg (MOV-LEG): the subjects will perform movements of the non amputated leg. Each participant will be instructed about the type and pace of the movements.
2. Movement of the leg observing the mirror (MIR-LEG): the subjects will perform the same movements from the previous condition, looking the mirror image of the intact leg in an online video. Each participant will be instructed about the type, pace and video.
3. Rest condition: the subjects will rest during this period and will be instructed to not perform any kind of movements during this period.

Each of these conditions will have the same length – 20 seconds. The fMRI session will have four runs that will contain 6 blocks (6 of each condition). The order of the conditions will be randomized. For the analysis we will determine the ROI of interest (primary sensory motor cortex of the lower limbs) and first determine the ratio of activation between the MOV-LEG and MIR-LEG (in the respective ROI of interest – contralateral to the respective leg). Using this coefficient we will then compare across the different conditions. Our hypothesis is that the combined group (tDCS and mirror therapy) will have a greater activation than any therapy alone

and also the no therapy group (sham tDCS and covered mirror). This effect will also be correlated with pain reduction.

VI. BIOSTATISTICAL ANALYSIS

Data forms and questionnaires will be coded in a standardized manner, and double-entered into our database. Digital measures/recordings will be similarly tracked in our database and regularly backed up. Analyses will be conducted using standard statistical software such as SAS and Matlab.

Sample size calculation:
To calculate the sample size for our primary outcome PLP as measured by a visual analogue scale (VAS), we used the study from Soler et al. as a reference ‘‘Effectiveness of transcranial direct current stimulation and visual illusion on neuropathic pain in spinal cord injury”. Using VAS as the basis for the calculation and considering results from this study: a mean and standard deviation of 5.2 (±1.5) in experimental group (tDCS + visual illusion) and 6.4 (±1.6) in control group (visual illusion), and using a bidirectional alpha of 0.05 and power of 80%, we would need a total sample of 108 subjects (27 subjects in each group). Additionally, estimating a conservative attrition rate of 20%, our sample size would be 132 subjects.
Although we calculated the sample for our primary aim, it is important to underscore that power calculation will be adequate also for our secondary aim measuring neurophysiological outcomes. In fact, neurophysiological outcomes usually have less variability thus need smaller sample sizes to show significant differences. In fact as shown in our preliminary data and also from data from other studies [58, 59] effect sizes from TMS data will be larger and for the fMRI, we expect BOLD changes in sensory motor cortex around 3 to 5% (as shown by previous studies [56]) – therefore a sample of 7 to 8 subjects will be enough to detect significant changes.

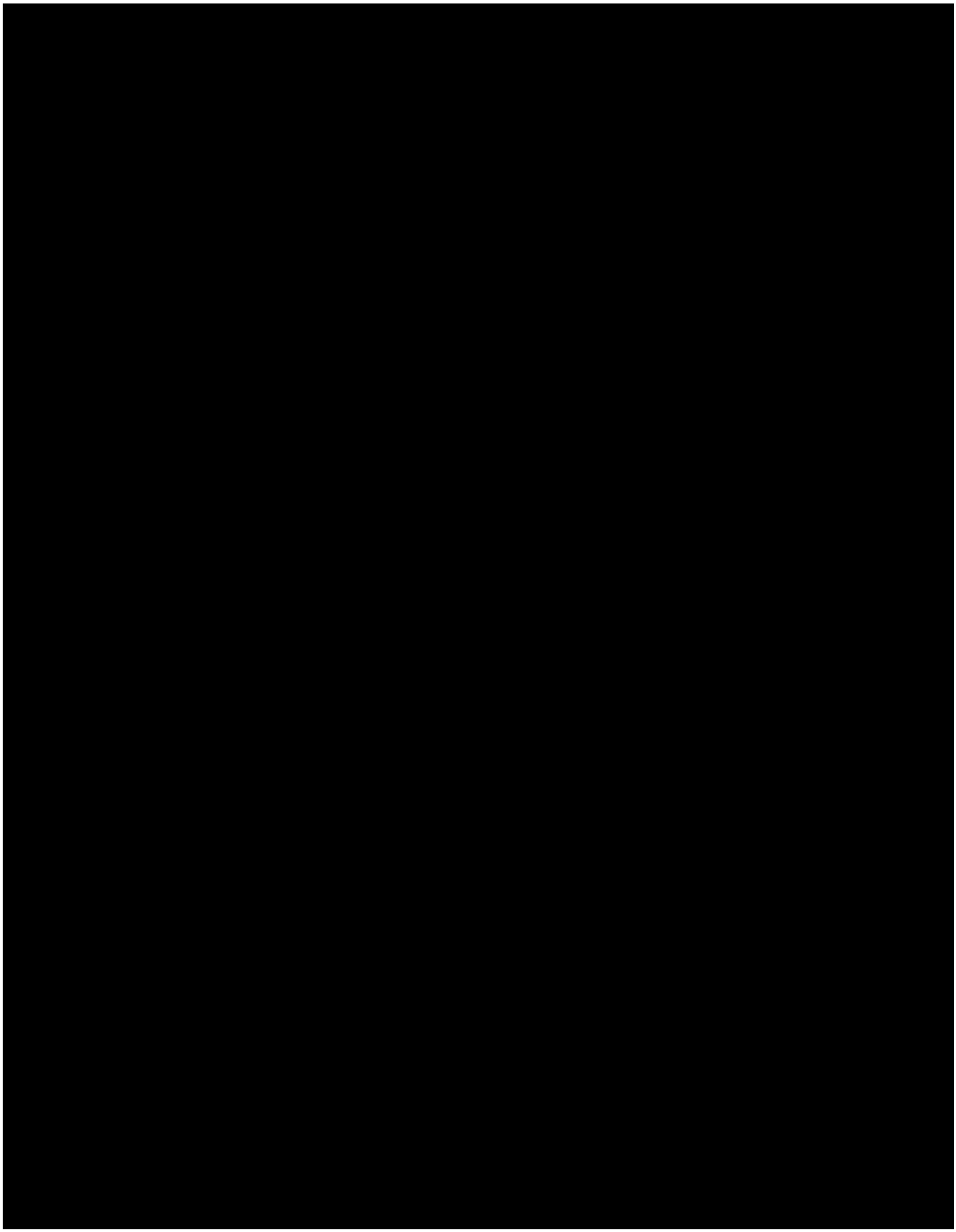
Statistical Analysis

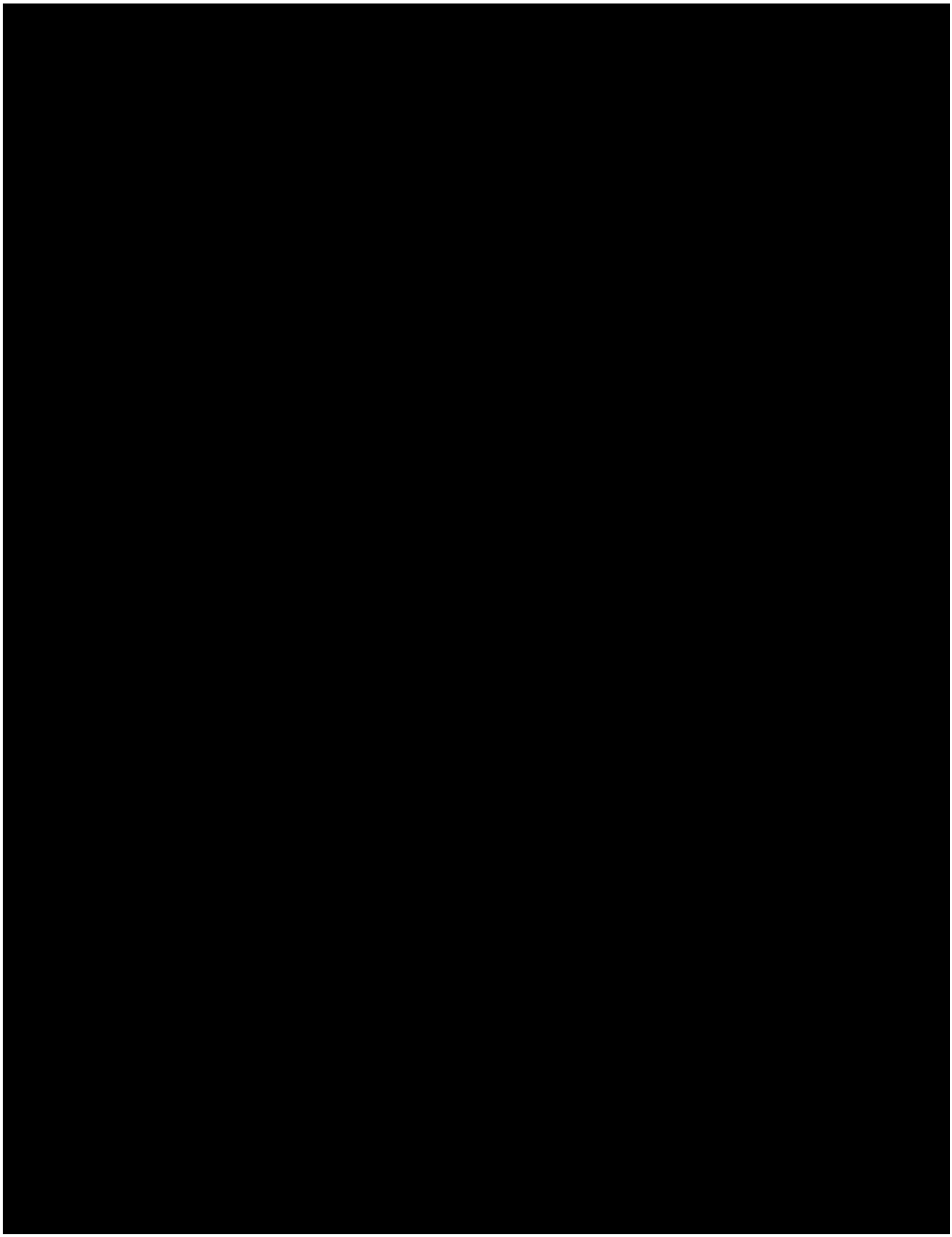
For aim 1:
The primary outcome is PLP indexed by VAS. PLP will be analyzed using intensity of pain over time. To analyze these data, we will initially build a mixed ANOVA model in which the dependent variable will be the outcome of PLP (such as VAS) and the independent variables will be group (active tDCS-MT; sham tDCS-MT; active tDCS and covered MT; sham tDCS- covered MT), time (baseline and after treatment and follow-up) and the interaction group*time. In addition we will add the random variable ID to account for within subject’s variability and the repeated measures on time. We will initially perform this full model and, subsequently, post-hoc comparisons with corrections for multiple comparisons will be carried out initially to test our main hypotheses. Furthermore we will test the correlation in improvement between outcomes using Pearson’s correlation tests. P-values for secondary and exploratory outcomes will be determined without corrections for multiple comparisons. Finally we will apply a path analysis [60, 61] to the primary outcome data to determine if pain reduction associated with the combined intervention (tDCS and MT) is due to direct effects versus indirect effects through improvement in secondary outcomes. We propose that a direct effect of tDCS and MT on pain can be assumed if the treatment effect cannot be explained by changes in psychological or functional outcomes. Statistical models for pain will be developed using covariates that include site center, baseline

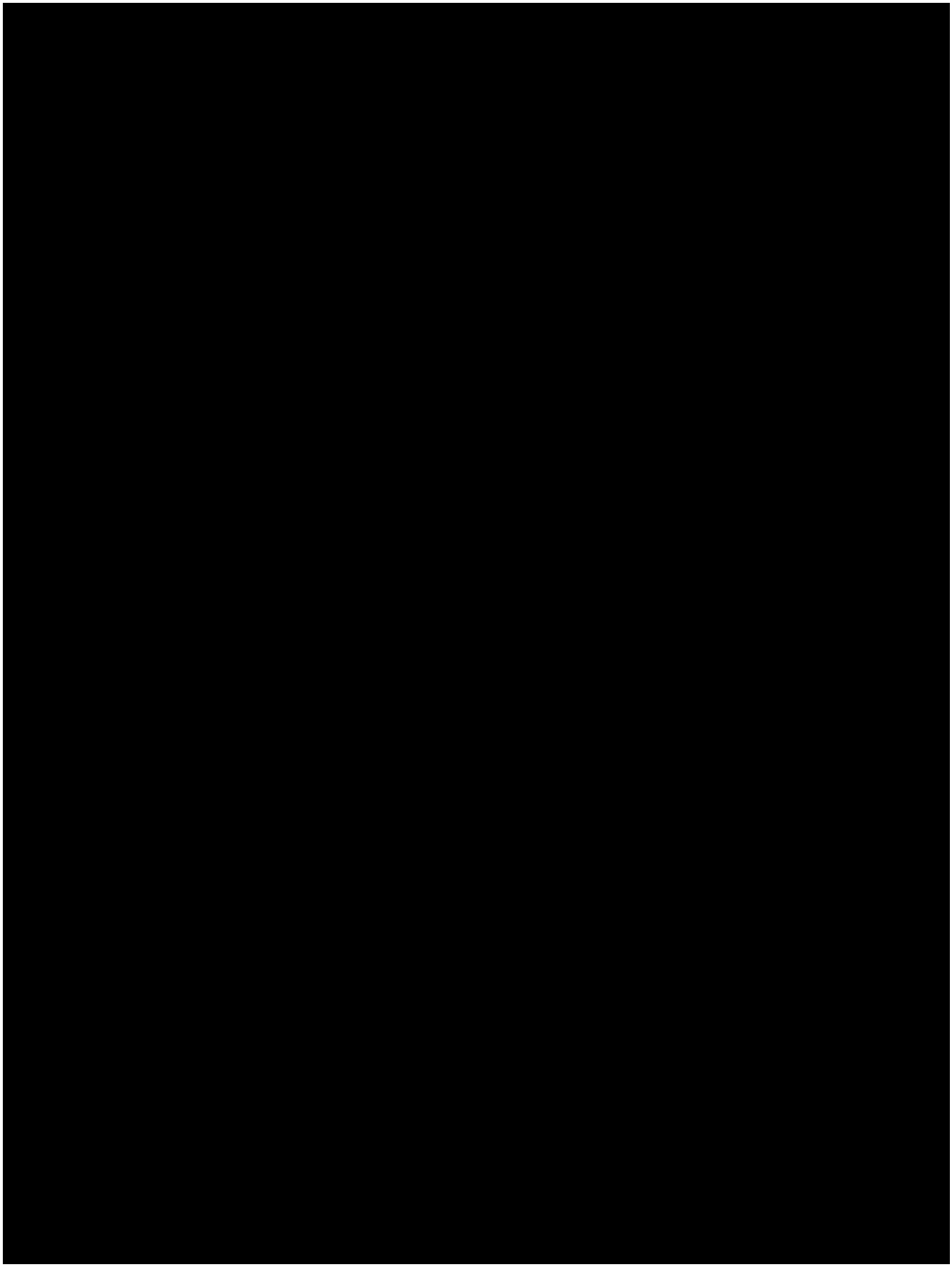
pain, psychological changes, functional changes, and the covariate treatment (main effect of treatment). To complete the path analysis separate regression models will be run to model the effects' of treatment on each outcome alone including all the secondary outcomes. Analyses of the secondary outcomes will be conducted in an exploratory manner (no correction for multiple comparisons). For the intention-to-treat analysis, we will use a conservative method and assume that participants will not improve from the last measured point. But we will also perform a sensitive analysis for the missing data using other methods such as completers only.

For aim 2:

The secondary outcomes are other pain measurements, psychological, neuropsychological and quality of life measurements and neurophysiological markers (as indexed by TMS and fMRI). Functional neuroimaging data will be analyzed using both Freesurfer image analysis suite combined with the FS-FAST functional data analysis package (Massachusetts General Hospital, Charlestown, MA) (http://surfer.nmr.mgh.harvard.edu/) and with Brain Voyager QX 1.9 software package (Brain Innovation, Maastricht, Netherlands). Briefly, a high resolution anatomical T1-weighted anatomical volume is used to reconstruct a cortical surface mesh and then inflated to allow viewing of sulcal activation. The functional preprocessing includes 3-D motion correction using the first volume alignment, high pass filtering to remove temporal linear trends, correction for slice time acquisition and spatial smoothing (Gaussian kernel, 5.0 mm FWHM). Subjects are screened for excessive motion (defined as a maximum tolerance of 3mm in any direction). Following co-registration of the preprocessed functional image with the high-resolution anatomical, data is transformed into standard Talairach space. Voxel time courses for each subject are fit by a general linear model (GLM) for statistical analysis. Each experimental condition is modeled by a boxcar regressor matching the condition time course. These boxcar regressors are then smoothed by a canonical hemodynamic response function [62]. Individual subject maps for each contrast of interest is generated by projecting the volume of significance values resulting from the GLM onto the reconstructed cortical surface mesh for each hemisphere in each subject. Statistical significance is set at a standard threshold criterion of $p<0.05$ corrected for multiple comparisons using a cluster-size threshold adjustment [63].







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