NYU School of Medicine
NYU LANGONE MEDICAL CENTER

Version Number	Version Date	Summary of Revisions Made
8.0	8 Dec 2020	• Option for healthy control subgroup to have an additional 15 minutes of neuroimaging combined with simultaneous tDCS (10 minutes) of up to 4.0 mA
7.0	10 June 2020	• Addition of a healthy control subgroup (n= 13)
6.0	19 NOVEMBER 2019	• BDI Screening over phone • Option to administer final visit questionnaires over phone • Clarification of implantation exclusion criteria
5.0	06 JUNE 2019	• Eligibility criteria for MS patients ○ BDI > 9 • Clarification of stop criteria • Addition of Ripple™

4.0	08 January 2019	• Study procedures for MS patients
		  ○ 2 MRI visits (baseline, treatment end)
		  ○ 3 month follow-up neuropsychological evaluation
		• Eligibility Criteria for MS patients
		  ○ Additional pre-screening measures
		○ Inclusion of Internal Study Monitor
		○ Healthy control recruitment from n= 20 to 25

3.0	27 NOVEMBER 2018	• Clarified the monetary compensation for MS participants

2.0	24 OCTOBER 2018	• Study procedures for MS patients
		  ○ Remotely-supervised tDCS (20 sessions)
		  ○ Treatment end, 3 month follow-up MRI visit Eligibility criteria
		• Endpoints
		• Eligibility Criteria
		• List of Mandatory and Optional Neuropsychological Assessments

1.0	06 JUNE 2018	Initial submission

NYU School of Medicine
NYU LANGONE MEDICAL CENTER

ASSESSMENT OF TDCS-INDUCED NEURONAL RESPONSES IN MULTIPLE SCLEROSIS (MS) WITH ADVANCED MRI

Principal Investigator:	Dr. Leigh Charvet, PhD Neurology 240 E38th Street, New York, NY 11211 Leigh.Charvet@nyumc.org 646-501-9257
Co-Principal Investigator:	Dr. Yulin Ge, MD Radiology 660 First Avenue, New York, NY 10016 Yulin.ge@nyumc.org 212-263-3784
NYULMC Study Number:	S18-00548

Initial version: 06/05/2018
Amended: 01/08/2019
Amended: 6/6/2019
Amended: 11/19/2019
Amended: 6/10/2020
Amended: 12/08/2020

Statement of Compliance

This study will be conducted in accordance with the Code of Federal Regulations on the Protection of Human Subjects (45 CFR Part 46), any other applicable US government research regulations, and institutional research policies and procedures. The Principal Investigator will assure that no deviation from, or changes to the protocol will take place without prior agreement from the sponsor and documented approval from the Institutional Review Board (IRB), except where necessary to eliminate an immediate hazard(s) to the trial participants. All personnel involved in the conduct of this study have completed Human Subjects Protection Training.

Table of Contents

List of Abbreviations

ACC	Ambulatory Care Center
AE	Adverse event/adverse experience
CMRO2	Cerebral metabolic rate of oxygen
CRF	Case report form
DLPFC	Dorsolateral prefrontal cortex
DSMB	Data and safety monitoring board
EKG	Electrocardiogram
fALFF	Fractional amplitude of low frequency fluctuations
FC	Functional connectivity
FDA	Food and drug association
GCP	Good clinical practice
HIPAA	Health insurance portability and accountability act
ICMJE	International committee of medical journal editors
MRI	Magnetic resonance imaging
MS	Multiple Sclerosis
MOP	Manual of procedures
MSCCC	MS Comprehensive Care Center
N	Number of study participants
NIH	National institute of health
NR	Neural reactivity
OHRP	Office of human research protection
PHI	Patient health information
PI	Principle investigator
QC	Quality control
RS-fMRI	Resting state functional MRI
SAE	Serious adverse event
SDMT	Symbol digit modalities test
SOP	Standard operating procedures
tDCS	Transcranial direct current stimulation
TRUST	T2-relaxation-under-spin-tagging
UP	Unanticipated problems
VMHC	Voxel-mirrored homotopic connectivity

Protocol Summary

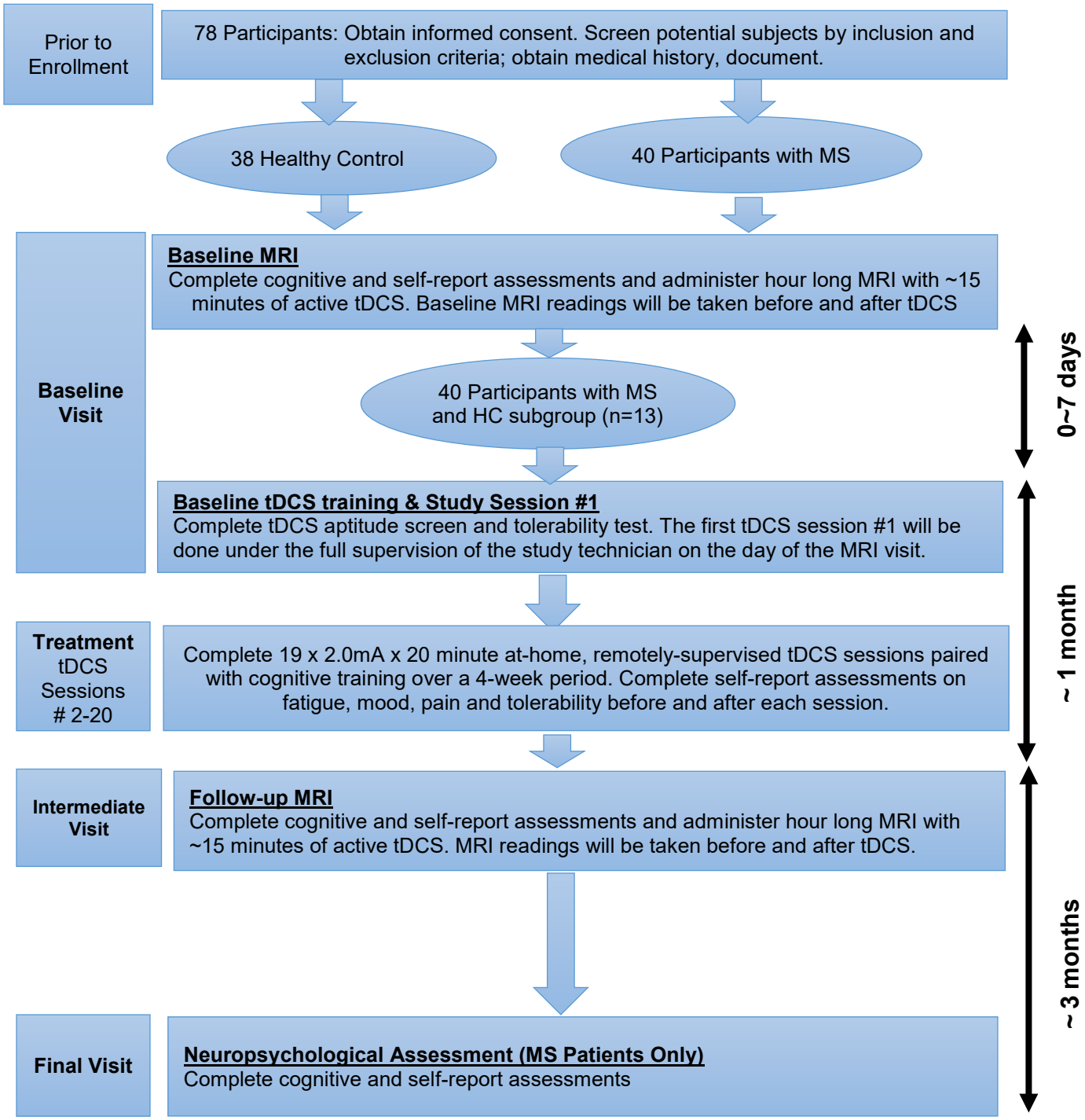
Title	Assessment of tDCS-Induced Neuronal Responses in Multiple Sclerosis (MS) with Advanced MRI
Short Title	tDCS-Neuroimaging Study
Brief Summary	Participants (N=78) (25 Healthy Controls (HC) plus subgroup of 13 HC for a total of 38 HC, and 40 clinically diagnosed MS patients) will be recruited to complete self-report measures and a brief cognitive assessment and then undergo an hour long stand-alone MRI scan while simultaneously undergoing tDCS stimulation. After the initial radiology visit, MS patients and the HC subgroup (n=13) will complete 20 remotely-supervised tDCS (RS-tDCS) sessions paired with cognitive training. MS patients will return for two follow-up visits: (1) MRI scan at the end of RS-tDCS treatment and (2) neuropsychological evaluation 3 months after the RS-tDCS treatment. The HC subgroup will return for the 1 month follow-up visit only.
Objectives	To test real-time (during tDCS), cumulative (treatment end) and lingering (3month) effects of tDCS in MS patients by correlating changes in neural activities (as assessed by MRI) with clinical outcome measures (as assessed from neuropsychological evaluation).
Methodology	Administration of around 15-minutes of active tDCS during MRI acquisition compared to time without active tDCS
Endpoint	1) Cerebral metabolic rate of oxygen (CMRO2) before and during tDCS. 2) Functional connectivity (FC): Functional networks of DLPFC will be extracted from pre-processed RS-fMRI data prepared within C-PAC toolbox. 3) Fractional Amplitude of Low Frequency Fluctuations (fALFF): fALFF is a RS-fMRI measure that quantifies the baseline neural activity by low frequency oscillations at rest.
Study Duration	This study will be ongoing for the next three years.
Participant Duration	For all subjects, each MRI visit will last for the duration of the MRI (approximately one hour) plus administration of self-report and cognitive assessments (approximately one hour) for a total of around 2 hours. 25 healthy subjects will only be asked to come in for a single MRI visit. 13 additional healthy controls will be asked to return for a 1-month follow-up. The HC subgroup participation will last approximately 1 month. For MS patients, the total duration will last 4 months which involves participation in (1) first MRI visit, (2) second MRI visit at the end of a treatment consisting of 20 at-home tDCS sessions (~ 1month), and (3) final neuropsychological evaluation 3 months after the treatment.
Duration of behavioral intervention	Approximately 15 minutes of active tDCS during one-hour imaging (for all). Optional for HC Subgroup: Approximately 25 minutes of active tDCS and 1 hour 15 minutes of imaging. Approximately 20 minutes of active tDCS during at-home tDCS daily sessions (for MS patients and HC subgroup).

Population	N = 78, 18 years of age or older: 40 individuals diagnosed with MS recruited from the MS Center/Neurology Department at NYULMC and the surrounding area and 38 healthy controls. No geographic location specifications as long as they are willing to pay for transportation to imaging center and are able to commit to the full hour scan.
Study Sites	NYULMC
Number of participants	78 participants
Description of Study Intervention/Procedure	Active tDCS up to 2.0 mA stimulation (with option for one-time use of up to 4.0mA for healthy control subgroup)
Reference Therapy	During radiological assessment, the measures obtained during the active tDCS portion will be compared to those obtained before when the stimulation was off.
Key Procedures	A pregnancy test will be given to any woman of child-bearing potential participating in the study prior to the MRI scan.
Statistical Analysis	This cross-sectional study will examine the real-time as well as the long-term effects of tDCS on neuronal reactivity in both HCs and patients with neurological deficits, as well as the long-term effects of tDCS treatment in MS patients. A p-value less than or equal to 0.05 will be considered significant for our paired-sample analyses.

Schematic of Study Design

Healthy Controls	tDCS + Neuroimaging (1 visit)	25
Healthy Control Subgroup	tDCS + Neuroimaging (2 visits; baseline & after treatment end) + 20 daily RS-tDCS sessions	13
MS Participants	tDCS + Neuroimaging (2 visits; baseline & after treatment end) + 20 daily RS-tDCS sessions + neuropsychological evaluation 3 months after treatment end	40

Flow Chart of Study Design



1 Key Roles

Leigh Charvet, PhD, Principal Investigator
NYU Langone Health — Neurology
240 E38th Street, New York, NY 11211
Leigh.Charvet@nyumc.org
646-501-9257

Yulin Ge, MD - Co-Principal Investigator
NYU Langone Health —Radiology
660 First Avenue, New York, NY 10016
Yulin.ge@nyumc.org
2120263-3784

Lauren Krupp, MD, Co-Investigator
NYU Langone Health — Neurology
240 E38th Street, New York, NY 11211
Lauren.Krupp@nyumc.org
646-501-7509

2 Introduction, Background Information and Scientific Rationale

2.1 Background Information and Relevant Literature

Transcranial direct current stimulation (tDCS) is a safe and noninvasive brain stimulation method that modulates cellular excitability by applying weak electrical currents (1 to 4mA) through electrodes resting on the scalp[1-3]. tDCS proves to be well tolerated up to a 4 mA stimulation with only minor irritation such as skin tingling and itching at the site of the anodal electrode[2,3]. tDCS can induce physiological neuroplasticity, which is crucial for functional reorganization and restoration. Patients with multiple sclerosis (MS), particularly with secondary progressive MS (SPMS), represent a key population for which tDCS can ameliorate major debilitating symptoms associated with their neurodegenerative condition, with particular promise in lessening fatigue[4,5] and enhancing cognitive function[6-8]. More pronounced improvement in fatigue[40,41] and cognitive function in MS patients was observed after multiple tDCS treatments (including our own that uses a remotely-supervised telemedicine protocol[8,9]). Despite its promising therapeutic potential[5,8-11], not all patients with MS respond to tDCS[12] and major gaps in knowledge of tDCS still exist regarding the underlying mechanism related to real-time effects as well as long-term consequences. This gap can directly affect the clinical evaluation and mechanistic interpretation of favorable and unfavorable responses that many practitioners are currently facing using tDCS as an alternative therapeutic approach.

MRI has had an enormous impact on MS, enabling non-invasive physiological measurements, elucidating disease mechanisms, and monitoring disease progression and therapeutic effects. We recently used a novel T2-relaxation-under-spin-tagging (TRUST)[13] MRI technique to measure the global cerebral metabolic rate of oxygen (CMRO2)[13,14], which demonstrated a significant decrease of O2 metabolism in people with MS[15]. CMRO2 provides a direct in vivo measure of neuronal cells' metabolic activity and vitality and is an ideal tool to assess neuronal reactivity (NR) (i.e., percent increase of CMRO2) when tDCS is applied as a neuronal stimulus. NR represents a potential biomarker of brain reserve that is important to predict tDCS outcome (i.e., responders vs. non-responders) on which little research has been completed. The task-based and resting state fMRI (RS-fMRI) studies[16-20] (including our own[21-23]) have shown disrupted functional connectivity in MS, which is considered to be responsible for various behavior and cognitive impairments. In this study, we will use anodal tDCS placed on the left dorsolateral prefrontal cortex (DLPFC) in participants to investigate neuronal responses to tDCS with advanced MRI techniques in real time. This will help characterize how externally applied electric current modulates brain oxygen metabolic physiology and functional connectivity (FC) through MRI.

To achieve this goal, we will use a state-of-the-art MRI-compatible tDCS design that permits simultaneous MRI acquisition during tDCS modulation[24-28], which is critical for true characterization of the real-time tDCS effects and for NR assessment. We will compare CMRO2 levels before and after stimulation with tDCS. To test the cumulative effects of tDCS, we will compare change in CMRO2 levels in MS patients before and after the completion of tDCS treatment. In addition, to test the lingering effects of tDCS, we will observe for a correlation between such CMRO2 level changes and improvements in clinical outcome measures (i.e. fatigue and neurocognitive function) assessed 3 months after the tDCS treatment. This work will be one of the few attempts to advance our understanding of tDCS's underlying neural mechanism using advanced MRI and to develop an objective neuroimaging biomarker (i.e., NR) for evaluating and predicting immediate, tDCS-induced neurophysiological changes.

2.2 Rationale

Combined with simultaneous MRI, the real-time tDCS will provide a powerful tool in assessing whole brain neuronal vitality and/or plastic potential (i.e. NR) and an individual's variability of clinical symptoms' improvement. Therefore, this protocol will add value by helping discriminate MS patients with favorable from unfavorable outcome after tDCS treatments and help develop person-specific tDCS protocols to maximize benefit for specific disease related deficits. The research design using MRI-compatible tDCS will also allow us to investigate the real time effects of tDCS. To date, one of the most troubling aspects of tDCS trials is that patients differ in how they respond to the brain stimulation treatment; and the cause of such differences remains unclear. The results generated from this project will, for the first time, highlight in vivo (by MRI) how baseline NR will predict any favorable or unfavorable clinical responses to tDCS. We predict the CMRO2 will increase after tDCS stimulation and remain elevated. The NR characterized by the percent increase of CMRO2 with tDCS represents the total amount of neuronal response that can be quantified by MRI. We also predict the baseline CMRO2 to be higher after multiple tDCS treatment sessions. Additionally, by performing MRIs on both non-healthy patients and Healthy Controls (HC), we will be able to uncover commonalities as well as differences in tDCS's effect on a healthy and non-healthy brain. This information will impact tDCS treatment strategies in MS and other disorders that may be evaluated for future clinical trials.

2.3 Potential Risks & Benefits

2.3.1 Known Potential Risks

Risks associated with MRI:
The MRI scans will be performed on a 3T Siemens scanner housed in the Bernard and Irene Schwartz Center for Biomedical Imaging (CBI) at NYU Langone Health. CBI is a research dedicated MRI facility that contains two 3T, one PET-MR (3T), and one human body 7T MR Siemens scanners. The MRI session will include the acquisition of conventional 3D T1-MPRAGE and FLAIR imaging as well as advanced MRI proposed in this study (detailed below) with total scan time approximately 60 minutes.

Magnetic Field and Imaging Risks: MRI scanning involves the use of a magnet and radio frequency waves (much like an ordinary short-wave radio). There are no known risks or adverse effects resulting directly from exposure to magnetic fields and radio frequency signals used in this study, other than the potential risks associated with the scanning procedure summarized below. These potential risks are present with any MRI procedure.

Implanted Devices: Subjects, who have pacemakers, certain aneurysm clips, or shrapnel fragments or persons with metal in the eye, are at risk for injury from MRI examinations.

Collision Hazard: Because of the strong magnetic field associated with the scanner, one risk is that of a metallic object flying through the air toward the scanner and hitting you. To reduce this risk, all people involved with this study will remove all metal from their clothing and all metal objects from their pockets when in the scanning environment.

Hearing Protection: The MRI scanner produces tapping sounds during operation, which may reach very loud levels. To minimize any discomfort from this noise, the subject will be provided with disposable earplugs that suppress external noise levels but do not eliminate voice communication with the scanner operator. Alternatively, in some cases, they will have headphones instead of earplugs, which will deliver other sounds.

Claustrophobia (Fear of confined spaces): Some people feel claustrophobic (fear of small spaces) in the MRI scanner. If this happens, the scan will be stopped immediately and the subject will be removed from the scanner.

Quench Hazard (MR system failure): The MR scanner uses liquid nitrogen and liquid helium. It is remotely possible that the liquid nitrogen and helium will boil off rapidly and fill the magnet room with extremely cold dense gaseous nitrogen and helium, which can be dangerous if breathed for more than a few moments. The scanner operator will obviously detect this and immediately provide assistance to anyone inside the magnet room.

During the MRI, subjects will be in visual and verbal contact with the experimenter throughout the scan through a video monitoring system and can be removed quickly. In addition, participants are given a squeeze ball so they can signal the scanner operator in the event of an emergency. Some subjects have experienced dizziness or a metallic taste if they move their heads rapidly in the magnet. This, however, is only temporary and does not occur if the head is still. Acoustical noise is generated by the charging and discharging of the gradient coils which create the magnetic fields used to generate an image. Subjects will be wearing hearing protection that reduces acoustic noise by approximately 30dB. All possible measures will be taken to educate research personnel concerning the dangers of metallic projectiles in the magnet room and any individuals entering the magnet room will be thoroughly screened for ferromagnetic material. The scanning session will last about 60 minutes.

Optional 15 minutes of additional MRI (healthy control subgroup only): an additional 15 minutes of imaging (1 hour 15 min total) poses minimal risk to participants and is equal to the MRI risks mentioned above.

Risks associated with tDCS:
There are no major risks associated with tDCS. Some people report head tingling, itchiness at the site of anodal stimulation, and a mild burning sensation. These irritations are usually mild and tolerable; however, some patients have expressed irritation that became painful forcing the abortion of tDCS session. These do not meet criteria for non-significant risk per Bikson et al., 2016. Additionally, there are no known serious adverse effects associated with this type of stimulation.

Optional tDCS up to 4.0mA (healthy control subgroup only): The safety and tolerability of higher tDCS current (up to 4.0mA) has been assessed in multiple studies[46,47,48] in which a single session of tDCS up to 4.0mA was found to be safe and well-tolerated.

2.3.2 Known Potential Benefits

There is no direct benefit to the participants expected from the tDCS protocol as established. It is expected that they have a personal medical doctor to provide medical care as needed and this study should not be used to supplement ongoing medical treatment for a chronic disease.

Incidental Findings
Incidental findings on MRI scans are possible health abnormalities that are found during the course of subjects' participation in this study and are unrelated to the research topic, but may be important for subjects and their physician to know about. Incidental findings that are identified by radiologists (all films including non-clinical research scans) may or may not have clinical significance as determined by the health professionals conducting this study will be communicated to Dr. Krupp, Co-I for this study, who will also review the neuroimaging reports.

If clinically useful information is uncovered, either the PI or another clinician on the study will speak to the subject in person or on the telephone regarding the new information. A copy of the original image report

will also be provided to the subject in person and subject will be encouraged to follow up on the discovery with their treating physician, outside of the study.

3 Objectives and Purpose

3.1 Primary Objective

The primary objective of this study is to accurately determine the real-time (during tDCS), cumulative (at the end of 20 treatment sessions), and lingering (3 months after treatment) effects of tDCS in MS patients by assessing changes in neural activities with stand-alone MRI and correlating such results with improvements in clinical symptoms i.e. fatigue and neurocognitive function. This finding will allow us to better understand tDCS's mechanism of action and further empower our field to leverage tDCS to create more precise changes to neurophysiology and ameliorate chronic symptoms in MS. Additionally, the proposed protocol will allow us to examine neurophysiological differences between tDCS administration in patients with neurological deficits and healthy control participants. This will allow our team to analyze whether tDCS effects are specific to healthy brain regions or if the stimulation can penetrate to areas of neural loss. Both objectives in this study will increase our understanding of tDCS and allow us to amend current treatment protocols to improve efficacy and specificity of treatment design.

3.2 Secondary Objectives

The secondary objective of this study is to relate demographic and clinical characteristics to MRI findings. This will allow us to relate a responder profile in MRI data to certain testing outcomes and better identify responders.

4 Study Design and Endpoints

4.1 Description of Study Design

MRI: The MRI scans will be performed on a 3T Siemens scanner housed in the Bernard and Irene Schwartz Center for Biomedical Imaging (CBI) at the NYU Langone Health. CBI is a research dedicated MRI facility that contains two 3T, one PET-MR (3T), and one human body 7T MR Siemens scanners. The MRI session will include the acquisition of conventional 3D T1-MPRAGE and FLAIR imaging as well as advanced MRI proposed in this study (detailed below) with total scan time approximately 60 minutes including the CMRO2 MRI discussed below.

Cerebral metabolic rate of oxygen (CMRO2) MRI: To quantify absolute CMRO2, both TRUST MRI for quantification of venous oxygenation (Yv) and phase contrast (PC) MRI for quantification of total blows are needed. The imaging details were described in our previous paper (Xu F et al, Magn Reson Med 2009). The total scan time for CMRO2 MRI is approximately 4 minutes.

Resting-state functional MRI (RS-fMRI): The sequence parameters for the rs-fMRI scans follow the ADNI protocol. We will use a 2D EPI sequence with SENSE partial-parallel imaging acceleration to obtain 3.3 × 3.3 mm (64 by 64 voxels) in plane resolution in forty-eight 3.3 mm transverse slices. An ascending slice order with TR/TE = 3000/30 ms, flip angle of 75°, and SENSE acceleration factor of 2 will be used. SPIR will be used for fat suppression. We will record 140 time points, for a scan time of 7 min.

This will be an open-label study, during which all participants including healthy controls and MS patients will be given approximately 15 minutes of active tDCS during MRI. The simultaneous tDCS will be performed up to 2 mA dose intensity to determine CMRO2 changes from when the tDCS is on to when the tDCS is off. The stimulation (left anodal at DLPFC regions) consists of 15-min up to 2 mA tDCS using 5x5 cm electrode sponges with ~30s ramp-up and ramp-down periods. MRI session includes 2 conditions: MRI-compatible tDCS off (immediately before and after tDCS) and active tDCS (in red frame) (refer to Figure 1). The RS-fMRI is performed before the active tDCS (with device turned off) to assess the network connectivity changes without any current influence. CMRO2 scans include PC MRI for CBF and TRUST MRI for Yv estimation; both sequences are not sensitive to the current-induced field inhomogeneity.

Optional: Participants in the healthy control subgroup will be given the option to have an additional 15 minutes of brain imaging, which will include 10 minutes of simultaneous tDCS at up to 4.0mA, added to the end of their baseline scan. If a participant opts-in, they will be asked to remain on the scanner for an additional 15 minutes after the initial MRI and tDCS procedure is complete.

After the first MRI visit, MS patients and HC subgroup will receive remotely-supervised home tDCS treatment for a month to test the cumulative effects of tDCS. Participants will complete 20 x 2.0mA x 20 minute tDCS sessions paired with cognitive training over 4-week period (20 mins per day and weekend off) with electrodes placed to target the bilateral DLPFC. Following our established clinical trial protocol[42,43], remotely supervised sessions will be completed while connected to a secure video conference session with the study technician (Vsee)[44]. The detailed procedures can be found in our recently published article[42]. Our protocol is opposed to self-directed home use, where a patient is given a device without parameters and real-time supervision, which is not advisable due to both safety concerns and problems regarding uniformity and reproducibility of results. Instead, we will maintain clinical trial standards for safety and consistency with a specially-designed tDCS device (that "unlocks" one "dose" per code, controlled by a study technician) extensive checkpoints and built-in safety features for the study with the utilization of remote supervision through a telemedicine videoconferencing platform[43]. Upon completion of 20 tDCS sessions, MS patients and the HC subgroup will be asked to come in for a follow-up MRI visit during which they will undergo the same hour-long MRI scan and neuropsychological assessments. Three months after the treatment end, MS patients only will come in for a final study visit which involves neuropsychological assessments only (participants who are unable to come in will complete the assessments over the phone). The study timeline is also described below in Figure 1.

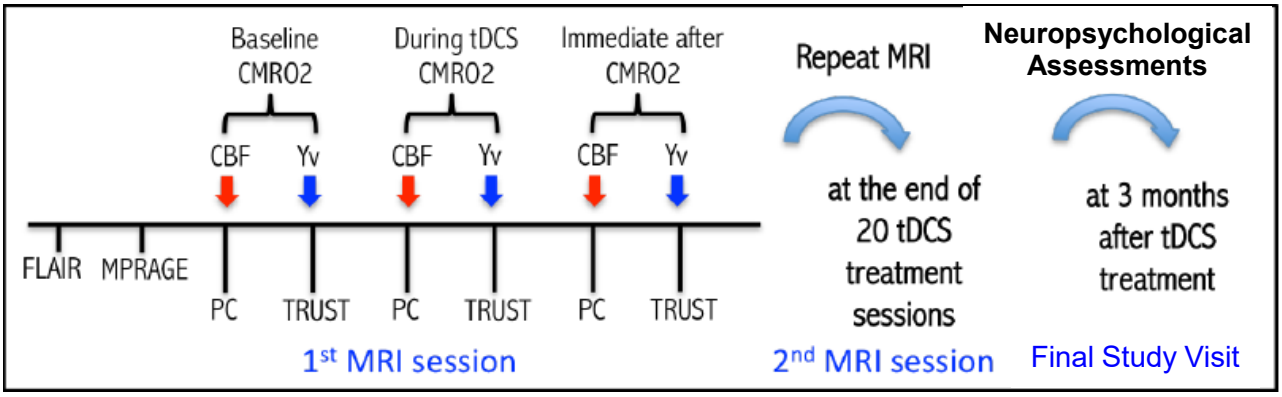


Figure 1. *Experimental protocol for MS participants*

4.2 Study Endpoints

4.2.1 Primary Study Endpoints:

The following imaging measurements will be performed with simultaneous tDCS with TRUST MRI and RS-fMRI. These measures will be compared between healthy controls and MS participants, and also across two different time points in MS participants and HC subgroup: 1) first MRI session and 2) at the end of a treatment consisting of 20 tDCS sessions (~ 1 month). We will assess a correlation between such change in imaging measures and outcome measures (fatigue and neurocognitive function) assessed 3 months after the treatment.

Imaging measures based on TRUST MRI:
Cerebral metabolic rate of oxygen (CMRO2): $CMRO_2$ is the amount of O_2 the brain consumes per unit of time (in µmol O_2/100g tissue per minute)[29,30]. Global CMRO2 will be measured with TRUST MRI before, during and immediately after tDCS stimulation in the scanner. Because most of the O_2 uptake is spent on neural functional activities, including the resting brain (e.g., cognitive functions), global $CMRO_2$ can be

viewed as a direct index of aggregated neural activities or viability of neuronal cells. When the blood is transported through capillary beds, a portion of the O_2 being carried is extracted by neural cells for metabolism, the rate of which is denoted by $CMRO_2$[31]. In this project we will use novel noninvasive methods to measure $CMRO_2$[14], by measuring venous oxygenation level (Y_v) with T2-relaxation-under-spin-tagging (TRUST) MRI and by measuring brain parenchyma CBF with phase-contrast (PC)[14,32] MRI. Y_v is quantified at the lower portion of the superior sagittal sinus (SSS), which is the largest venous sinus draining the most cerebral cortex blood. Y_a is the oxygenation level in arteries and is determined by the pulse oximetry. Once these three parameters are known, $CMRO_2$ can be calculated using Fick's principal. Neuronal Reactivity (NR): NR represents tDCS-induced global neural reactivity measured by $CMRO_2$ levels in cells available to respond to neural stimuli. Once we have CMRO2 measurements as described above, with our MRI-compatible tDCS, NR is measured as the percentage change of $CMRO_2$ from no tDCS to real tDCS (up to 2.0 mA for ~ 15 min) scan. The global NR represents the amount of neuronal potential to a neuronal stimulus. In MS, reduced NR can exacerbate the onset and severity of disability[33,34] because the intricate potential for pathological and physiological plasticity[6,35] is determined by NR, an important indicator of cell reactivation and network reorganization. Compared to the task-based fMRI, tDCS-induced NR evaluation is paradigm-free and uses externally dose controlled (passive) stimulation, and is more reliable and suitable for people with difficulty performing the cognitive tasks.

Imaging measures based on RS-fMRI:
Functional connectivity (FC): Functional networks of DLPFC will be extracted from pre-processed RS-fMRI data prepared within C-PAC toolbox. Left DLPFC is defined as the seed (10 mm radius sphere) chosen as the location using the MNI coordinates (centered at x, y, z of −42, 34,30) [36]. FC between DLPFC and other brain regions uses the seed-based correlation analysis (SCA) to generate z-score correlation (Fisher's r-to- z transformation) maps, which is the one of the most common ways to explore functional network[37]. Specifically, DLPFC network maps will be defined by functional clusters reaching a contiguous volume of at least 176m at a voxel-wise threshold of $p<0.001$ (one sample t-test) that is considered at corrected $p<0.05$, and will make use of the subsequent two-sample t-tests.

Fractional Amplitude of Low Frequency Fluctuations (fALFF) [38]: fALFF [38] is a RS-fMRI measure that quantifies the baseline neural activity by low frequency oscillations at rest. fALFF of each voxel is defined as the ratio of power spectrum of low frequency (0.01–0.08 Hz) range to that of the entire frequency range compute, and then the fALFF value of each voxel became normalized through dividing the global mean fALFF value. Finally, fALFF on the post-hoc DLPFC network maps will be used for comparison between the two MRI visits. Decreased ALFF and fALFF in the DLPFC regions has been reported in MS patients.

Voxel-mirrored homotopic connectivity (VMHC)[39]. VMHC characterizes synchrony in patterns of spontaneous activity between symmetric homotopic (geometrically corresponding) cortical regions (e.g., DLPFC) in each hemisphere. VMHC maps were generated by calculating the correlation strength between each voxel and its counterpart on the opposite hemisphere after registration to a symmetric template brain, and the variation of VMHC strength is thought to reflect hemispheric and regional specialization in information processing.

4.2.2 Secondary Study Endpoints

Secondary study endpoints will link behavioral measures of quality of life and cognition to tDCS neuronal response.

4.2.3 Exploratory Endpoints

At the discretion of study staff, clinical characteristics such as MS lesion load, degree of brain atrophy and DLPFC volume will be obtained from conventional 3D T1-MPRAGE and FLAIR imaging. Exploratory endpoints will link baseline demographic and clinical characteristics to test whether individual differences in tDCS response can be predicted by these features.

Exploration of extended dosing ranges from the standard one (2.0mA) is important for identification of maximally efficient stimulation approaches. To address this, neuronal response to tDCS up to 4.0mA will

be assessed using simultaneous quantitative MRI measures proposed in this study but will only take 15 minutes.

5 Study Enrollment and Withdrawal

5.1 Inclusion Criteria for MS Patients

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

1. Participants must be 18 years of age or older
2. Standardized SDMT Z-score > - 3.0
3. Fatigue Severity Scale score > 36
4. Definite MS diagnosis as assessed by licensed physician any subtype including Relapsing Remitting (RRMS), Primary Progressive (PPMS) or Secondary Progressive (SPMS)
5. Score of ≤ 7.0 on the Expanded Disability Status Scale
6. Clinically stable without disease progression in the past 3 months
7. Has stable and continuous access to internet service at home compatible with the study laptop (Wi-Fi or Ethernet cable)
8. Adequate internet capacity for remote monitoring, as tested by http://www.speedtest.net/
9. Adequate home facilities (enough space, access to quiet and distraction free area)
10. Able to commit to the four-week period of training sessions with baseline and two follow-up visits

5.2 Exclusion Criteria for MS Patients

In order to be eligible to participate in this study, an individual must not have a history of the following:

1. Extreme claustrophobia
2. Relapse or steroid use in previous month
3. History of mental retardation, pervasive developmental disorder, or other neurological condition associated with cognitive impairment
4. Primary psychiatric disorder that would influence ability to participate
5. Current uncontrolled seizure disorder
6. Current substance abuse disorder
7. Any skin disorder/sensitive skin (e.g., eczema, severe rashes), blisters, open wounds, burn including sunburns, cuts or irritation, or other skin defects which compromise the integrity of the skin at or near stimulation locations (where electrodes are placed)
8. Treatment for a communicable skin disorder currently or over the past 12 months
9. Have any irremovable piercings, MRI-contraindicated implantations or metallic based-tattoos
10. Pregnant or breastfeeding
11. Wide Range Achievement Test-4th Edition (WRAT-4) Reading Recognition Scaled Score < 85
12. Beck Depression Inventory – Fast Screen (BDI-FS) score > 9

5.3 Inclusion Criteria for Healthy Controls

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

1. Participants must be 18 years of age or older
2. Standardized SDMT Z-score > - 3.0
3. Have not been diagnosed with MS or other neurological disorder
4. Adequate internet capacity for remote monitoring, as tested by http://www.speedtest.net/

(HC subgroup only)

5. Adequate home facilities including enough space, access to quiet and distraction free area **(HC subgroup only)**
6. Able to commit to the four-week period of training sessions with baseline and one follow-up visit **(HC subgroup only)**

5.4 Exclusion Criteria for Healthy Controls

In order to be eligible to participate in this study, an individual must not have a history of the following:

1. Extreme claustrophobia

2. History of mental retardation, pervasive developmental disorder or other neurological condition associated with cognitive impairment
3. Primary psychiatric disorder that would influence ability to participate
4. Current uncontrolled seizure disorder
5. Current substance abuse disorder
6. Any skin disorder/sensitive skin (e.g., eczema, severe rashes), blisters, open wounds, burn including sunburns, cuts or irritation, or other skin defects which compromise the integrity of the skin at or near stimulation locations (where electrodes are placed)
7. Treatment for a communicable skin disorder currently or over the past 12 months
8. Have any irremovable piercings, MRI-contraindicated implantations or metallic based-tattoos
9. Pregnant or breastfeeding

5.5 Vulnerable Subjects

We will be including NYU employees and undergraduate/graduate students as healthy controls. These healthy controls will neither be given any additional incentives to participate in the study including for students, incentives to increase their academic standings, nor will their academic standing or grades be negatively impacted if they choose not to participate or participate and later withdraw from the study. Participation or refusal to participate will have no impact on employees' employment/position, salary or job performance.

5.6 Strategies for Recruitment and Retention

The MS Comprehensive Care Center (MSCCC) of NYU Langone Health has an extensive recruitment base. Patients will be recruited to participate in studies from all over New York tri-state area and anywhere within the United States. Patients who are seen by medical staff at NYU Langone Health, who fit the eligibility criteria, will be referred for the study by the study PI and sub-investigators. All physicians and medical staff at the MS Care Center will be presented with the study description. A patient who is seeing one of these medical staff members as their treating physician will be introduced to the study by that medical staff member. If the patient is interested and agrees, then a member of the study staff will contact them. Once a patient is identified, study staff will meet with the patient or call them to provide additional information regarding study participation. After the patient has reviewed the consent form and asked all questions, and provides consent to participate, the patient will be enrolled in the study.

An IRB approved flyer will be posted in local physician offices and waiting rooms and throughout NYU, the surrounding community and support organizations. In addition to recruitment at NYU's MSCCC, we will post these flyers around the ACC for patients and healthy controls to recruit from the entirety of the Neurology department and from any number of people who come to visit the Ambulatory Care Center (ACC) on E38th Street.

Healthy controls will be recruited through friends and family of employees at the MS Center, NYU students, employees at NYULMC by word of mouth.

A study description will be posted on MS related websites and websites specific to the healthy control age range we are reaching out too.

Consent will be administered prior to the MRI scan while at the imaging center.

All potential participants will complete a telephone pre-screen to ensure general eligibility. The study staff member speaking to the subject will provide the subject with an overview of the study using IRB approved telephone script and verbally receive their permission, under a waiver of documentation of consent, to complete the general eligibility screening. This phone screen is minimal risk to the participant and collected information will be maintained in secured, locked files. De-identified information (assigned a study screening code) will be entered into a secure, excel spreadsheet on the NYU server in a file specific to this study accessible only by IRB approved staff members. If a participant is not eligible, they will be considered a screen fail. No additional information will be collected. PHI will be destroyed immediately if a

participant is not eligible or does not return to sign written consent/authorization to participate. Only study staff will have access to these records.

Participant personal information may also be kept in Ripple™, a secure web application designed for the storing and management of personally identifying information of research participants. Ripple was initially developed at the University of Michigan to provide a user friendly, web-based secure interface where research teams can centralize the storage and management of research participants' personal information, including name, participant ID, demographics, and study workflow (e.g., appointments). Participant information managed with ripple is private and secure. This information is kept in fully encrypted format inside dedicated databases that are segregated from other Ripple accounts and thus only authorized study staff will have access to the study data. Likewise, Ripple infrastructure complies with the privacy and security guidelines of the Health Insurance Portability and Accountability Act (HIPAA), including 2048-bit data encryption in transit and at rest, automatic logoff, audit trail, daily backups in triplicate dedicated servers, firewall, custom access permission for lab members, zxcvbn password strength estimation, and enterprise administrative safeguards to prevent unauthorized staff from accessing participant information. Furthermore, Ripple is used only for storing personally identifiable information of participants and is not used to capture other research data (e.g., questionnaires, health records, etc.). This ensures that the personally identifiable information and research data are segregated. Ripple is already being used at NYU Langone Health.

Once the participant is generally eligible, the PI, or one of the trained study team members will review the consent form with the subject and explain the purpose of the study, the procedures, as well as risks and benefits. All questions will be addressed before acquiring the participant's signed consent.

5.7 Duration of Study Participation

All participants will complete a single baseline MRI visit that can take up to two hours and thirty minutes (or 2 hours and 45 minutes for participants in the HC subgroup participants who opt-in to additional 15 min scan +tDCS) in total. The visit will include a single hour MRI session as well as about an hour of cognitive assessments and self-report questionnaires. Additionally, preparation for the MRI will be roughly 20 minutes, including the eligibility screen and MRI prep. This MRI preparation includes undressing to get into the medical robe, taking a urine pregnancy test, speaking with the MRI study team member about the scan and commencing the scan.

Following the above initial visit, MS patients and the HC subgroup will receive 20 remotely-supervised tDCS (RS-tDCS) sessions (~1month). MS patients and the HC subgroup will return for a radiology visit at the end of RS-tDCS treatment during which they will repeat the same neuropsychological assessments and an hour long MRI scan conducted on the baseline visit.

MS patients will return for neuropsychological assessments 3 months after the RS-tDCS treatment. The final visit may be completed over the phone if participant is unable to come to clinic.

5.8 Total Number of Participants and Sites

78 participants will be recruited for the study (25 HC plus a HC subgroup of n=13, and 40 MS). Target enrollment for the healthy control subgroup is 10, however 13 may be enrolled to account for screen failures and withdrawals. Recruitment will end when approximately 75 participants completed their required study procedures. It is expected that approximately 78 participants will be enrolled in order to produce 75 evaluable participants.

Sites include NYU's MSCCC and 38th Street Ambulatory Care Center for recruitment, however, imaging will only be done at the Center of Biomedical Imaging (CBI) located on 38th Street and First Avenue.

5.9 Participant Withdrawal or Termination

5.9.1 Reasons for Withdrawal or Termination

Participants are free to withdraw from participation in the study at any time upon request. An investigator may terminate participation in the study if:

- Any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant

5.9.2 Premature Termination or Suspension of Study

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to Dr. Charvet. If the study is prematurely terminated or suspended, the PI will promptly inform the IRB and will provide the reason(s) for the termination or suspension.
Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants as determined by pain monitoring questionnaires administered during the MRI scan.
- Demonstration of inefficacy that would warrant stopping
- Insufficient compliance to protocol requirements as assessed during safety monitoring meetings, PI Dr. Leigh Charvet, MD-CoI Dr. Lauren Krupp and Internal Study Monitor Dr. Zhovtis-Ryerson.
- Data that are not sufficiently complete and/or evaluable

Study may resume once concerns about safety, protocol compliance, data quality are addressed and satisfy the sponsor and/or IRB.

6 Behavioral/Social Intervention

6.1 Study Behavioral or Social Intervention(s) Description

tDCS will be applied for approximately 15 minutes to 20 minutes during this study. tDCS can induce both pathological and physiological neuroplasticity, which is crucial for functional reorganization and restoration. In this proposed project, we will investigate how brain metabolism and network connectivity properties are modulated by anodal tDCS.

6.1.1 Administration of Intervention

tDCS Administration during MRI:

The MRI compatible tDCS will be administered for a single session during MRI scan. The device used will be the MRI compatible tDCS device by Soterix medical https://soterixmedical.com/research/1x1/tdcs. The Soterix 1x1 tDCS device provides the direct current via anode and cathode electrodes. A wire resistant to the MRI machine's magnetism is attached to the tDCS device outside of the room in the wiring panel and passes through a RF filter before entering the room. The magnetism has no effect on the current delivered to the participant in the MRI room and has been extensively assessed by Soterix engineers.

The device will produce up to 2.0 mA stimulation for around 15 minutes during the MRI session. The stimulation will be provided at a tolerated level determined by a tolerability test, provided prior to MRI commencement, going up to 2.0 mA. The set-up of the device and administration of stimulation will be done by an onsite study team member from the Charvet lab. The Study team member will correctly place the headset and control the delivery of stimulation.

Optional 15 minutes of imaging + simultaneous tDCS up to 4.0mA: Participants in the healthy control subgroup who are willing may have an additional 15 minutes of imaging combined with 10 minutes of

simultaneous tDCS of up to 4.0mA added at the end of their baseline scan. In this case, after the initial imaging and tDCS portion described above is complete, the participant will remain on the scanner for an additional 15 minutes during which brain imaging will continue and will be combined with 10 minutes of simultaneous tDCS up to 4.0mA. This will be a one-time add-on and will not be required at follow-up.

Remotely-supervised tDCS Administration:

All remotely-supervised sessions will be completed while connected to a video session with the study technician. The remotely-supervised protocol is designed to have a decision-tree series of checkpoints that must be met in order to proceed at each step (Figure 2). These checkpoints address compliance (attendance, ability to complete the procedures as instructed, following the study guidelines) and tolerability (at any time, if any predefined events are reported). Adverse events will be documented through to resolution, and the severity and need to report to the IRB will be assessed by the study PI.

For at-home tDCS administration, participants will be given the specially-designed tDCS device and headset, study laptop computer for secure video monitoring with study technician (must have internet access) and access to the cognitive training program, a detailed reference manual, and a training video. The Soterix 1x1[45] is uniquely designed for remotely-supervised delivery and requires a one-time use code provided by the study technician to unlock the device for one stimulation session. The device will produce up to 2.0 mA stimulation for around 20 minutes, and will not operate without the correct headset placement. The device will also automatically abort the session if optimal conditions are not maintained. It reports and records a completion code for each session.

While the first tDCS session will be performed at clinic under the full supervision, remaining sessions 2-20 will be completed at home with remote monitoring by the study technician. Participants in the HC subgroup who opt-in to the additional 15 minutes of imaging +tDCS may complete the first session remotely.

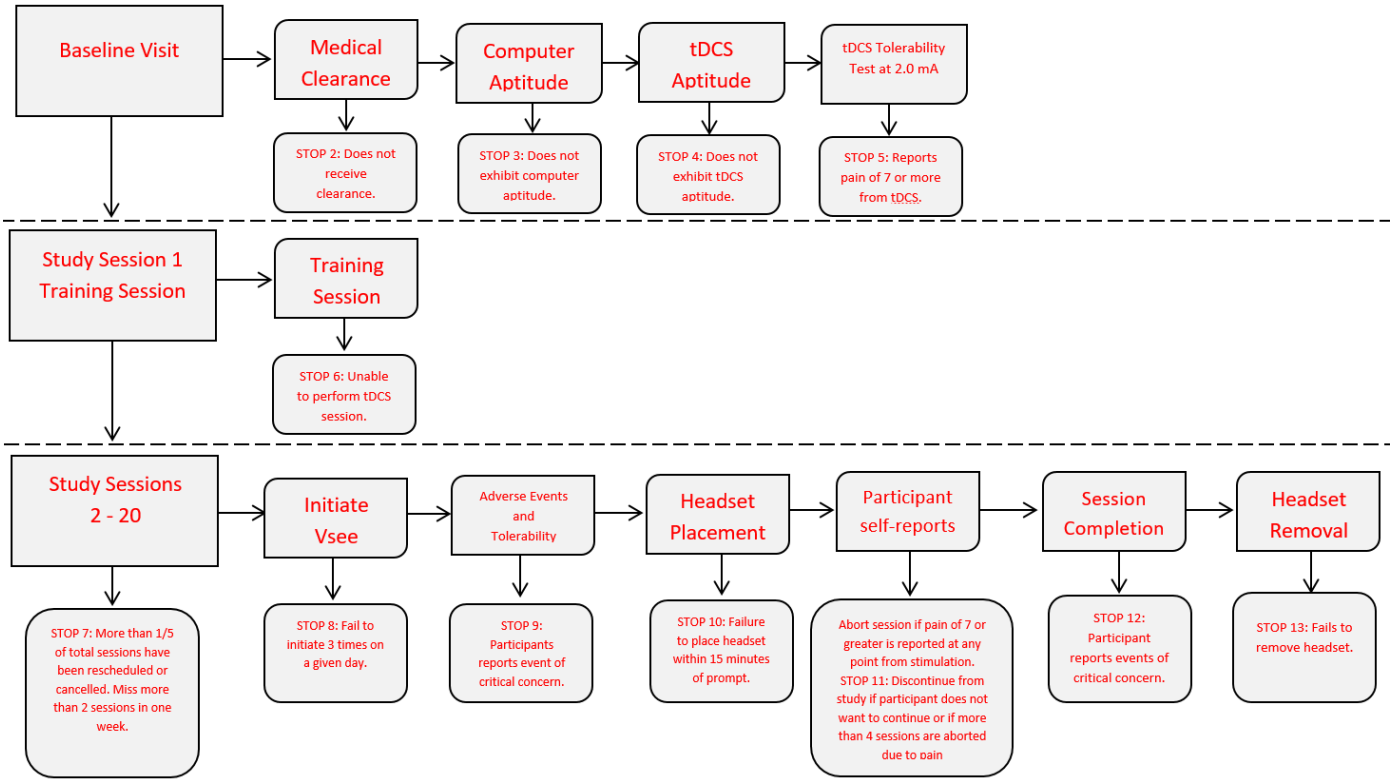
Participants will schedule times during which they are certain they can self-administer the tDCS while they are being remotely monitored by study staff. They will be observed using a secure internet-based video chat program that will be installed in the laptop they will use for the study. To start their session, the participant will connect to study staff via a secure internet-based video program. They will put on the tDCS headgear while being monitored, and tell study staff if the device feedback indicates that the electrodes are acceptably placed. The participant will then receive the activation code from the tDCS device. If the study staff observes the participant making any errors that may cause the latter discomfort they can intervene with instructions for correction.

Safety provisions:
The tDCS device can only operate if:

1) The headset is correctly placed for adequate connection, and
2) The study technician provides a session code that unlocks the device for a one-time only 20 minute period of use.

If the device loses adequate contact for any reason, the device will automatically discontinue the session. The session can only be reestablished if another unlock code is provided by the study technician.
If the participant wishes to discontinue the session at any time, they will be instructed to press the “abort” key which ramps down the current within 30 seconds to allow for headset removal.

Figure 2. *Stop Criteria in Remotely-Supervised tDCS Protocol for MS patients*



*If a participant experiences a pain of 7 or greater, the supervising study technician will immediately intervene to assess the subject's safety and abort the session when necessary.

6.1.2 Procedures for Training Interventionists and Monitoring Intervention Fidelity

Study team members have been trained to monitor patients and healthy controls undergoing tDCS. The study team members ensure correct placement of the headset to minimize pain associated with improper headset placement (e.g. placement over the trigeminal nerve). Additionally, the study team members will ask about pain level from the headset at numerous points throughout the session and will always be listening to the participants during the session in case they express distress related to the headset.

Participants will be informed that if pain levels are 7 or greater on a standard pain scale, to immediately notify the study team member so safety can be evaluated and, if necessary, the study session stopped. If a participant seems to be distressed at any point (i.e. verbal cues, physical discomfort, etc.) during MRI scan, the study team member will have the capacity to contact the patient using the MRI intercom. During the MRI, there is audio and visual monitoring of the participant to supplement all check-in procedures. If a participants wishes to discontinue at any time during the remotely-supervised tDCS session, he/she will be instructed to press the single-button "abort" key which ramps down the current within 30 seconds to allow for headset removal.

6.1.3 Assessment of Subject Compliance with Study Intervention*

No compliance questionnaires will be necessary for this study.

7 Study Procedures and Schedule

7.1 Study Procedures/Evaluations

A potential participant will be phoned and scheduled to come to one of the study sites at NYU Langone Health for an agreed upon day and time. During the phone call and after receiving verbal consent from the potential participant, the participant will be screened for eligibility criteria as to not have the participant come to the study site and have to be sent home due to non-eligible status. For MS participants, baseline screening evaluations will include a documentation of a neurological exam and medical clearance by the study physician, and the ability to operate the study equipment for remotely-supervised tDCS sessions. Once the participant arrives at the imaging site, they will redress in scanning clothes, take off removable jewelry, and place in a locker for safe keeping during the scan.

After enrollment, MS participants and HC subgroup will have 2 MRI sessions (Figure 1): (1) the first starting session and (2) at the end of 20 tDCS treatment (~1month). Around 3 months after the 20-session treatment, MS patients only will be asked to come to the clinic for neuropsychological assessments. Twenty-five healthy controls will have only 1 MRI visit. Once the scan commences the protocol will follow the study design shown in Figure 1 below. Ultimately, the CMRO2 measurement is simplified as before-then-active-then-after- stimulation in the scanner using the MRI-compatible tDCS device to characterize both real time and residual effects of tDCS modulation. Each MRI session includes 3 conditions (1) tDCS off (before stimulation), (2) real tDCS, and (3) tDCS off (after stimulation). Active tDCS is to mimic the length of a treatment session that is commonly used in the literature. Both CMRO2 and RS-fMRI measures are acquired during tDCS stimulation as well as immediately after when the tDCS is turned off.

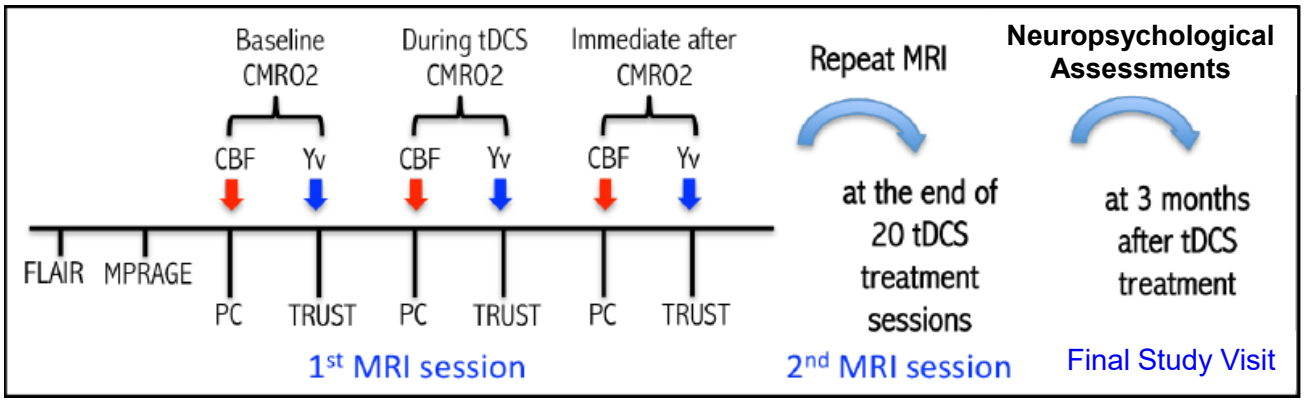


***Figure 1**. Experimental protocol for MS participants*

7.1.1 Clinical Laboratory Evaluations

A pregnancy test will be given prior to the start of the MRI for women of child-bearing potential. Once the participant is confirmed to not be pregnant the study will continue.

7.1.2 Cognitive Assessments and Self-report Questionnaires

Before and after tDCS administration, participants will complete cognitive assessments and self-report questionnaires related to fatigue, mood, pain and tolerability. A group of cognitive assessments and self-reports will be mandatory for every participant while others will be optional and performed at the discretion of the study staff to give to participants. Similarly, some self-report questionnaires are designed for MS patients and are not applicable for healthy controls; we will specify which questionnaires and measures are applicable only to the MS cohort. See Table 1 for a full breakdown of which cognitive assessments and self-reports will be administered to participants.

Table 1. Mandatory, Optional and Applicable Cognitive Assessments and Self-Report Questionnaires

Measure
tDCS Session Questionnaire *
Visual Analogue Pain and Fatigue Scale *
Positive and Negative Affect Schedule (PANAS) *
Cognition Lab Processing Battery †
Attention Network Test-Interaction (ANTI) †
Symbols Digits Modalities Test (SDMT) †
Wide Range Achievement Test (WRAT-4) †
Neuro Quality of Life †
Test of Everyday Cognitive Ability †
Beck Depression Inventory – Fast Screen †
Positive and Negative Affect Schedule †
Controlled Oral World Association Test – Verbal Fluency †
Rey Auditory Verbal Learning Test †
Brief Visuospatial Memory Test – Revised †
Fatigue Severity Scale (FSS) ††
Pittsburgh Fatigability Scale ††
Multiple Sclerosis Neuropsychological Questionnaire ††
Modified Fatigue Impact Scale ††
Tasks marked with an asterisk (*) are mandatory and must be completed by all participants before and after each tDCS session. Tasks marked with a cross (†) are optional and be completed by participants either before or after tDCs administration at the discretion of study staff. Tasks marked with a double cross (‡‡) are optional for MS participants only.

7.2 Study Schedule

After the participant has been screened using the eligibility questionnaire, we will set up a date and time for them to come to the imaging center. Once they arrive at the imaging center, the participant will be asked to disrobe and place belongings in a locker for safe keeping and put on a patient robe. At this point the participant will be given an informed consent by a study team member from the MS Center and the MRI Safety Questionnaire will be administered per standard protocol for radiology procedures. If applicable, the patient will be given a pregnancy test. If the test is negative, the participant will be directed into the MRI-tDCS simultaneous scanner and begin the study. They will be monitored by study team members for the entirety of the MRI-tDCS scan which will last for roughly one hour. At the conclusion of the MRI, the participant will be paid for their participation and given the option to view their scans.

After the above radiology visit, MS patients and the HC subgroup will receive training to undergo a month of remotely-supervised tDCS treatment. They will be asked to come in for a follow-up visit after the completion of the at-home tDCS treatment. MS patients will be asked to come in for 3rd visit 3 months after the completion of remotely-supervised tDCS. MS patients who are unable to come to clinic for the final follow-up may complete the assessments over the phone.

7.2.1 Screening

Screening for eligibility criteria can happen over the phone with verbal consent of the participant (which is a pre-screen) or in person before the completion of the informed consent form. Pre-screening measures including SDMT, WRAT-4, FSS & BDI will be done over the phone prior to enrollment. If pre-screen measures are completed on the same day as the baseline visit, these screening measures may be used as the baseline measures. Exclusion and inclusion criteria for the study are listed below. Before enrollment, participants must meet the relevant eligibility criteria depending on their condition type (MS patient or healthy control).

.

Inclusion/Exclusion Criteria for MS Patients

Inclusion Criteria (From IRB approved protocol)	Yes	No	Supporting Documentation*
Ages 18-79	☐	☐	.....
Standardized SDMT Z-scores > -3.0	☐	☐	.....
Fatigue Severity Scale score > 36	☐	☐	
Able to understand all study instructions and supply written consent	☐	☐	.....
Definite MS diagnosis as assessed by licensed physician any subtype including Relapsing Remitting (RRMS), Primary Progressive (PPMS) or Secondary Progressive (SPMS)	☐	☐	.....
Score of ≤ 7.0 on the Expanded Disability Status Scale (EDSS)	☐	☐	
Clinically stable without disease progression in the past 3 months	☐	☐	
Has stable and continuous access to internet service at home compatible with the study laptop (Wi-Fi or Ethernet cable)	☐	☐	
Adequate internet capacity for remote monitoring as tested by http:// www.speedtest.net/	☐	☐	
Adequate home facilities (enough space, access to quiet and distraction free area)	☐	☐	
Able to commit to a month of tDCS training sessions as well as two follow-up visits	☐	☐	

Exclusion Criteria (From IRB approved protocol)	Yes	No	Supporting Documentation*
Extreme claustrophobia	☐	☐	.....
Relapse or steroid use in previous month	☐	☐	
History of mental retardation, pervasive developmental disorder or other neurological condition associated with cognitive impairment	☐	☐	.....
Primary psychiatric disorder that would influence ability to participate	☐	☐	.....
Current uncontrolled seizure disorder	☐	☐	.....
History of head trauma in the past year (e.g., head injury, brain surgery) or medical device implanted in the head (such as Deep Brain Stimulator) or in the neck (such as a Vagus Nerve Stimulator)	☐	☐	.....

Any skin disorder/sensitive skin (e.g., eczema, severe rashes), blisters, open wounds, burn including sunburns, cuts or irritation, or other skin defects which compromise the integrity of the skin at or near stimulation locations (where electrodes are placed)	☐	☐	.....
Treatment for a communicable skin disorder currently or over the past 12 months	☐	☐	.....
Have any irremovable piercings, MRI contraindicated implantations or metallic-based tattoos	☐	☐	.....
Pregnant or breastfeeding	☐	☐	.....
Current substance abuse disorder	☐	☐	.....
WRAT-4 Reading Recognition Scaled Score < 85	☐	☐	
BDI – FS score > 9	☐	☐	

Inclusion Criteria for Healthy Controls (n=25)

Inclusion Criteria (From IRB approved protocol)	Yes	No	Supporting Documentation*
Ages 18-79	☐	☐	.....
Standardized SDMT Z-scores > -3.0	☐	☐	.....
Able to understand all study instructions and supply written consent	☐	☐	.....
Have not been diagnosed with MS or other neurological disorder.	☐	☐	.....

Inclusion Criteria for Healthy Control Subgroup (n=13)

Inclusion Criteria (From IRB approved protocol)	Yes	No	Supporting Documentation*
Ages 18-79	☐	☐	.....
Standardized SDMT Z-scores > -3.0	☐	☐	.....
Able to understand all study instructions and supply written consent	☐	☐	.....
Have not been diagnosed with MS or other neurological disorder.	☐	☐	.....
Has stable and continuous access to internet service at home compatible with the study laptop (Wi-Fi or Ethernet cable)	☐	☐	
Adequate internet capacity for remote monitoring as tested by http://www.speedtest.net/	☐	☐	
Adequate home facilities (enough space, access to quiet and distraction free area)	☐	☐	
Able to commit to a month of tDCS training sessions as well as two follow-up visits	☐	☐	

Exclusion Criteria for all Healthy Controls (From IRB approved protocol)	Yes	No	Supporting Documentation*
Extreme claustrophobia	☐	☐	.....
History of mental retardation, pervasive developmental disorder or other neurological condition associated with cognitive impairment	☐	☐	.....

Primary psychiatric disorder that would influence ability to participate	☐	☐	.....
Current uncontrolled seizure disorder	☐	☐	.....
History of head trauma in the past year (e.g., head injury, brain surgery) or medical device implanted in the head (such as Deep Brain Stimulator) or in the neck (such as a Vagus Nerve Stimulator)	☐	☐	.....
Any skin disorder/sensitive skin (e.g., eczema, severe rashes), blisters, open wounds, burn including sunburns, cuts or irritation, or other skin defects which compromise the integrity of the skin at or near stimulation locations (where electrodes are placed)	☐	☐	.....
Treatment for a communicable skin disorder currently or over the past 12 months	☐	☐	.....
Have any irremovable piercings, MRI contraindicated implantations or metallic-based tattoos	☐	☐	.....
Pregnant or breastfeeding	☐	☐	.....
Current substance abuse disorder	☐	☐	.....

7.2.2 Enrollment/ Baseline

For MS participants, baseline screening evaluations will include a neurological exam and medical clearance by the study physician. The study physician will complete the following:

- Neurological examination
- Medical history
- Medication history
- Physical examination

*** Note clinician assessment for medical clearance may occur either before or on the day of baseline MRI visit**

Baseline MRI Visit (all participants)

- Obtain informed consent of potential participant verified by signature on study informed consent form.
- Verify inclusion/exclusion criteria.
- Obtain urine pregnancy test for women who are of child-bearing age
- Administer assessments related to cognition and fatigue.
- Administer MRI safety questionnaire.
- Give the participant a tDCS tolerability assessment including 90 seconds at full stimulation level.
- Administer the MRI-tDCS scan.
- Optional for HC Subgroup only: a one-time add-on of additional 15 minutes of brain imaging combined with simultaneous 10 minutes of tDCS of up to 4.0mA added to the end of their scan

Baseline tDCS training visit (MS participants and HC subgroup)

Participants will be evaluated on their ability to independently operate the study equipment by completing the tDCS aptitude screen and tolerability test.

- tDCS Aptitude: participants will first complete an aptitude test to confirm that they have the cognitive and motor skills required for headset placement. With the instruction of the study technician, they will be asked to insert the sponges onto the headset and place the electrodes into the sponges. The technician will determine whether or not the participant is qualified to

proceed. Participants will not be allowed to proceed if they are not able to correctly either 1) attach sponges to headset or 2) place electrodes in the sponges

- tDS tolerability: The study technician will next directly place the headset and then initiate a one-minute test session, with 30 seconds of ramp-up to target, followed by a 30-second ramp down. The tolerability test will first take place using 2.0 mA stimulation. If the participant cannot tolerate the stimulation level, they are excluded from the study.

Optional for HC Subgroup: Participants in the HC subgroup who opt-in to the additional 15 minutes of imaging with simultaneous tDCS (10 minutes) will have a tolerability test at 4.0 mA. If 4.0mA is not well tolerated (as indicated by participant) the tDCS will be decreased in 0.5 increments (e.g 3.5mA, 3.0mA, 2.5mA) until the participant's mA tolerability level is reached.

Participants who successfully passed the tDCS aptitude screen and tolerability test will complete the first tDCS session in clinic while the remaining 19 sessions would occur from home with remote supervision.

***Note that baseline MRI and tDCS training visits can take place on different dates. The participants will be compensated for a separate tDCS-training visit which can occur within 7 days after the baseline MRI visit.**

7.2.3 Intermediate sessions (for MS participants and HC subgroup)

Remotely- supervised at-home tDCS treatment sessions 2 – 20 (~ 1month)
Each session will involve:

- Administration of tDCS at 2.0 mA while performing cognitive training
- Pre and post- session measures of fatigue, mood, pain and tolerability
- Participants in the HC Subgroup who opt-in to the additional imaging+tDCS may complete the first session remotely after session instruction is provided by team member.

7.2.4 Intermediate Visit (MS participants and HC subgroup)

MRI Visit (within 1 week from the end of the treatment +/- 7 days)
Participants will be asked to come in for a follow-up MRI visit at the end of their 20 sessions of remotely-supervised tDCS treatment i.e. around 1 month from the beginning of 20 sessions.

- Verify inclusion/exclusion criteria.
- Obtain urine pregnancy test.
- Administer assessments related to cognition and fatigue.
- Administer MRI safety questionnaire.
- Administer the MRI-tDCS scan

7.2.5 Final Study Visit (MS participants)

Neuropsychological Assessments (3 months post-treatment +/- 14 days)
MS participants will be asked to come in to complete assessments related to cognition and fatigue around 3 months after the completion of remotely-supervised tDCS treatment i.e. around 4 months from the beginning of 20 sessions. If a participant is unable to come to clinic for the final visit, the assessments will be administered over the phone. In this case, research compensation will be mailed to participant.
See Table 2 for a summary timeline of study procedures.

	All participants		MS participants and HC subgroup		MS participants
Test	**Screen**	**Baseline Visit**	**Daily tDCS Sessions (2-20)**	**Intermediate Visit (1 month ± 1 week)**	**Final Visit (4 month ± 2 weeks)**
Medical Clearance *	X				
Expanded Disability Status Scale *	X				
tDCS Aptitude Test and Training *		X			
tDCS Tolerance Test (2.0 mA) (or up to 4.0mA for HC Subgroup who opt-in)		X			
MRI Safety Screening Questionnaire		X		X	
MRI Scan		X		X	
tDCS Pre and Post- Session Questionnaire		X		X	
Daily tDCS Pre and Post- Session Questionnaire		X	X		
Visual Analogue Pain and Fatigue Scale		X	X	X	X
Positive and Negative Affect Schedule (PANAS)		X	X	X	X
Cognition Lab Processing Battery †		X		X	X
Attention Network Test-Interaction (ANTI) †		X		X	X
Symbols Digits Modalities Test (SDMT) †	X	X		X	X
Wide Range Achievement Test (WRAT-4) †	X*	X		X	X
Neuro Quality of Life †		X		X	X
Test of Everyday Cognitive Ability †		X		X	X
Beck Depression Inventory – Fast Screen †	X*	X		X	X
Positive and Negative Affect Schedule †		X		X	X
Verbal Fluency †		X		X	X
Rey Auditory Verbal Learning Test †		X		X	X
Brief Visuospatial Memory Test – Revised †		X		X	X
Fatigue Severity Scale (FSS) † *	X*	X		X	X
Pittsburgh Fatigability Scale † *		X		X	X
Multiple Sclerosis Neuropsychological Questionnaire † *		X		X	X
Modified Fatigue Impact Scale † *		X		X	X
Score for computerized cognitive games (daily) †			X		
Daily assessment of sleep † *			X		
Count of successful tDCS sessions			X		
Tasks marked with an asterisk (*) are for MS patients only. Tasks marked with a cross (†) are optional and be completed by participants either before or after tDCs administration at the discretion of study staff.					

Table 2. *Summary timeline of study procedures*

8 Assessment of Safety

8.1 Specification of Safety Parameters

tDCS Administration during MRI:

tDCS tolerability will be assessed prior to tDCS-MRI. If stop is requested during the MRI for any reason, tDCS-related or not, the session will be immediately aborted. Additionally, if the pain level ever reaches at

or above a 7 due to tDCS stimulation (as in the tolerability test), the study technician will immediately assess patient safety and abort the tDCS stimulation when necessary. Participant monitoring will be possible due to audio-visual monitoring at the imaging center. Stop criteria can be viewed below (Figure 3).

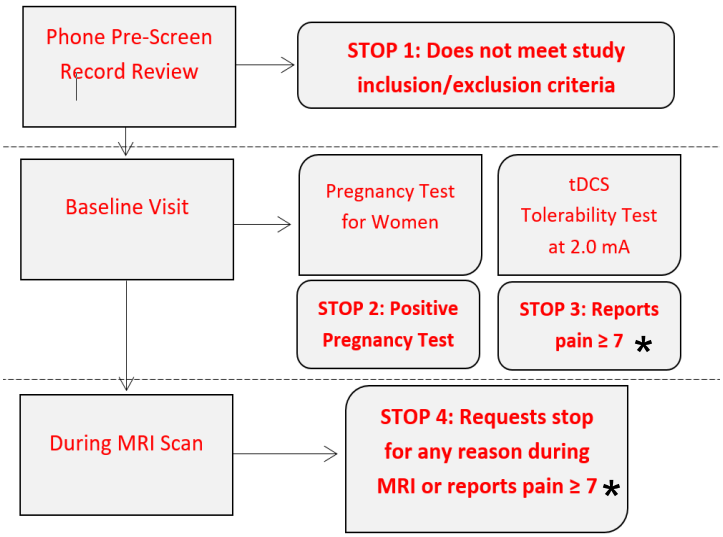


Figure 3. *Stop Criteria during MRI-tDCS combined session*

* If a participant experiences a pain of 7 or greater, the supervising study technician will immediately intervene to assess the subject's safety and abort the session when necessary

Remotely-supervised tDCS Administration:

As described under 6.1.1 Administration of Intervention, several safety parameters exist to ensure the safety of MS participants during their participation in remotely-supervised, at-home tDCS sessions (Figure 2).

8.1.1 Definition of Adverse Events (AE)

An ***adverse event*** (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Inter current illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

8.1.2 Definition of Serious Adverse Events (SAE)

Serious Adverse Event

Adverse events are classified as serious or non-serious. A ***serious adverse event*** is any AE that is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious should be regarded as ***non-serious adverse events***.

8.1.3 Definition of Unanticipated Problems (UP)

Unanticipated Problems Involving Risk to Subjects or Others
Any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in nature, severity, or frequency (i.e. not described in study-related documents such as the IRB-approved protocol or consent form, the investigators brochure, etc)
- Related or possibly related to participation in the research (i.e. possibly related means there is a reasonable possibility that the incident experience, or outcome may have been caused by the procedures involved in the research)
- Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, or social harm).

8.2 Classification of an Adverse Event

8.2.1 Severity of Event

For AEs not included in the protocol defined grading system, the following guidelines will be used to describe severity.

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating.

8.2.2 Relationship to Study Intervention

The study PI, in conjunction with CO-I and clinicians, will determine the relationship of any adverse events or unanticipated problems to study procedures and equipment.

8.2.3 Expectedness

The study PI will be responsible for determining whether an AE is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention.

8.3 Time Period and Frequency for Event Assessment and Follow-Up

The occurrence of an AE or SAE may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor. All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate RF. Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study intervention (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE. UPs will be recorded in the data collection system throughout the study.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

The PI will record all reportable events with start dates occurring any time after informed consent is obtained until participant completes study. Dr. Krupp will be informed of the occurrence of AEs/SAEs, review the AE/SAE with the PI and study team, and assess the event based on institutional guidelines regarding relatedness to the study, harm to the patient, or if it is expected. . Additionally, at each study meeting, the PI will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

All unresolved adverse events should be followed by the PI as well as MD-Co Investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The investigator should notify the study sponsor of any death or adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study.

8.4 Reporting Procedures – Notifying the IRB

8.4.1 Adverse Event Reporting

All adverse events will be reported to IRB per NYU policy.

8.4.2 Serious Adverse Event Reporting

All serious adverse events will be reported to IRB per NYU policy. Presence of an SAE will be included on data safety monitoring reports to the IRB and an immediate report will be submitted to the IRB within 48 hours of the event occurring. Dr. Charvet will review all SAE reports prior to their submission and follow up with each patient.

8.4.3 Unanticipated Problem Reporting

Incidents or events that meet the OHRP criteria for UPs require the creation and completion of an UP report form. It is the site investigator's responsibility to report UPs to their IRB. The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- UPs that are SAEs will be reported to the IRB within 10 days of the investigator becoming aware of the event.
- All UPs should be reported to appropriate institutional officials (as required by an institution's written reporting procedures), the supporting agency head (or designee), and OHRP within10 days of the IR's receipt of the report of the problem from the investigator.

8.4.4 Reporting of Pregnancy

In the case of a positive pregnancy test, the participant will be informed of the test results and excluded from participating in the study.

8.5 Study Halting Rules

If there are more than two SAEs of similar intensity we will halt study enrollment and further examine our protocol. Safety review will be undertaken by the Charvet lab group and Dr. Lauren Krupp, a medically licensed investigator with expertise in Multiple Sclerosis, to assess the protocol and amend as necessary. Post-review, a modified protocol must be submitted to the IRB addressing SAEs and how amended protocol addresses the issue.

8.6 Safety Oversight

It is the responsibility of the Principal Investigator to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan. Medical monitoring will include a regular assessment of the number and type of serious adverse events with the advice of our MD-Co Investigator, Dr. Lauren Krupp and Internal Study Monitor, Dr. Zhovtis-Ryerson. Safety oversight will be under the direction of a data safety monitoring board (DSMB) composed of Study team members trained in the protocol (all study team members submitted to IRB) and who can be considered tDCS experts (Dr. Charvet). Additionally the DSMB will include radiologists (Dr. Ge) and neurologists (Dr. Krupp and Dr. Zhovtis-Ryerson). The DSMB will meet at least quarterly to discuss each participant who is enrolled in the study and have completed the MRI and discuss any noteworthy occurrences for each participant and how those occurrences or issues were resolved. The DSMB will submit study data safety monitoring reports to the IRB annually unless increased risk/safety issues occur. Dr. Charvet and Dr. Krupp as well as Dr. Zhovtis-Ryerson will review all AE reports prior to their submission.

9 Clinical Monitoring

Not Applicable

10 Statistical Considerations

10.1 Statistical Hypotheses

We hypothesize that NR levels will be highest during the active tDCS portion of the study as compared to when tDCS is turned off. Additionally, we expect that NR will be higher in HC participants rather than participants with neurological conditions.

We also hypothesize that after completion of 20 tDCS treatment sessions, MS participants will have higher baseline (without simultaneous tDCS) $CMRO_2$ than the baseline $CMRO_2$ measured during the first MRI session.

10.2 Description of Statistical Methods.

10.2.1 General Approach

This cross-sectional study will examine the effects of tDCS on NR in both HCs and patients with neurological deficits across multiple time points. A p-value less than or equal to 0.05 will be considered significant for our paired-sample analyses.

10.2.2 Analysis of the Primary Efficacy Endpoint(s)

Paired sample t-tests will be performed to analyze the change in NR from baseline NR level to tDCS activity level. Repeated measures analysis of covariance will be used to compare the CMRO2 levels at the selected times adjusting for subject-level factors such as age and lesion load. The dependent variable will be the

vector of $CMRO_2$ at all available times for all patients. The NR and $CMRO_2$ values will be aggregated via preexisting data generation algorithms to be generated by staff at the imaging center.

10.2.3 Analysis of the Secondary Endpoint(s)

Analysis of secondary study endpoints will correlate self-report mood, fatigue and sleep and cognition scores to tDCS-induced neuronal response as assessed by change in $CMRO_2$ levels. Analysis will be done using a Pearson's correlation. This will help to characterize whether person-to-person characteristics correlates with tDCS responsiveness and if non-tDCS related neural activity correlates with personal characteristics. tDCS responsiveness is defined as having presented improvement in at least one of measures (self-report mood, fatigue, sleep, cognition scores) after tDCS treatment.

10.2.4 Safety Analyses

We will check in with the participant at various points during tDCS session and ask them to rate their pain levels on a 0-10 scale. This will ensure that the tDCS is being well tolerated and the participant is comfortable.

10.2.5 Baseline Descriptive Statistics

Baseline statistics will include age, race, ethnicity, years of education, disease diagnosis (if applicable), and date of diagnosis/disease duration (if applicable).

10.2.6 Planned Interim Analysis

10.2.6.1 Safety Review

If a participant reports a pain level at or above a 7 on the 0-10 pain scale, the participant's safety will be evaluated and, if necessary, the study session stopped. Additionally, the MRI scan will be stopped if the participant requests abortion of the scan for any reason including claustrophobia, any unforeseen form of discomfort, non-responsive to intercom questions.

10.2.6.2 Efficacy Review

Aggregate MRI measures will be compiled for active tDCS section and non-active tDCS portion of the MRI. A paired sample t-test will be done to assess the mean difference between MRI measures during active and non-active comparing both sub-groups of HCs and non-healthy participants.

10.3 Sample Size

The sample size will be 75 participants (78 total enrollment to account for screen failures and withdrawals). 35 participants will be healthy controls (25+10 HC subgroup) and 40 participants will have a multiple sclerosis diagnosis. Sample size is determined by need for robust data for statistical analysis and adequate healthy controls to have age and gender matched data points. Age and gender matched controls will be used as a baseline to analyze NR in participants with neurological conditions that lack robust sample size.

10.4 Measures to Minimize Bias

10.4.1 Enrollment/Randomization/Masking Procedures

There is only a single arm for this study. During the MRI scan, all participants in the study will receive the same treatment, 15 minutes of inactive tDCS followed by around 15 minutes of active tDCS. The participant will be told that during the study there will be a period of around 15 minutes when the tDCS will be turned on and that rest of the time the tDCS will be off. Thus, the participant will be fully informed of the protocol as will be the study team members.

During remotely-supervised tDCS sessions, all MS participants and 13 healthy control subgroup will receive 20 minutes of active tDCS conditioned paired with cognitive remediation.

11 Source Documents and Access to Source Data/Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A". All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. DO NOT ERASE OR WHITE OUT ERRORS. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it.

Access to study records will be limited to IRB-approved members of the study team. The investigator will permit study-related monitoring, audits, and inspections by the IRB/EC, the sponsor, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

12 Quality Assurance and Quality Control

QC procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

- Development of standard protocols to perform all data collection and follow-up activities.
- Use of standardized forms.
- Uniform criteria for patient recruitment.
- Standardized data processing.
- Regular communications between study staff and study investigators to resolve questions.
- Performance monitoring of data collection and data processing activities, as well as preparation of periodic reports and analyses on performance monitoring.
- Monthly monitoring of recruitment statistics

13 Ethics/Protection of Human Subjects

13.1 Ethical Standard

Our goal is to elucidate potential neurological mechanisms to promote more effective treatments for tDCS. As described, across hundreds of clinical trials, including in MS and our pilot studies, tDCS has been found to be safe and well-tolerated. A recent and comprehensive safety review found no risk of adverse events with tDCS as planned for use in this study [25]. All potential participants will be informed of any possible side effect. Prior to enrollment, participants will undergo medical clearance with a confirmation of enrollment criteria including cognitive screening measures to ensure that they have sufficient cognitive capacity to understand the fundamental principles of clinical research, the specifics of this study, and to comply with study procedures.

Dr. Charvet's focus of her career has been to measure and ameliorate the symptoms of Multiple Sclerosis including cognitive impairment and fatigue. She is a world leader in remote-supervised transcranial direct current stimulation (RS-tDCS) including several publications on safety and feasibility. She brings over 20 years of clinical research experience to this project, including key investigator roles in past NIH-supported projects. She is a licensed clinical neuropsychologist and training has included the completion of an ABPP-ABCN certified internship and postdoctoral fellowship programs in clinical neuropsychology with both adult and pediatric training.

The investigator will ensure that this study is conducted in full conformity with Regulations for the Protection of Human Subjects of Research codified in 45 CFR Part 46.

13.2 Institutional Review Board

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the consent form will be IRB approved; a determination will be made regarding whether previously consented participants need to be re-consented.

13.3 Informed Consent Process

13.3.1 Consent/Assent and Other Informational Documents Provided to Participants

Consent forms describing in detail the study intervention, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to starting intervention. The informed consent form and documentation of consent materials are submitted with this protocol.

13.3.2 Consent Procedures and Documentation

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Extensive discussion of risks and possible benefits of participation will be provided to the participants and their families. Consent forms will be IRB-approved and the participant will be asked to read and review the document. The investigator will explain the research study to the participant and answer any questions that may arise. All participants will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participant will sign the informed consent document prior to any procedures being done specifically for the study. The participants may withdraw consent at any time throughout the course of the trial. A copy of the signed informed consent document will be given to the participants for their records. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

A copy of the signed informed consent document will be stored in the subject's research record. The consent process, including the name of the individual obtaining consent, will be thoroughly documented in the subject's research record. Any alteration to the standard consent process (e.g. use of an interepreter consent document presented orally, etc.) and the justification for such alteration will likewise be documented.

13.4 Participant and Data Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why

- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, their data will be removed as soon as subject revokes authorization to PHI.

Participant confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

The study monitor or representatives of the IRB may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by local IRB and Institutional regulations.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the MS Comprehensive Care Center. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The subjects name and personal information will never appear on any of the images/data attained during the course of the study. Their consent form and any identifying information will be locked in a separate cabinet from the imaging data all in the MSCCC at the ACC at 222 E41st Street. The IRB approved study personnel will only have access. The study data entry (NYU RedCap database) and study management systems (Research Navigator, CRMS) used by clinical sites and by MS Comprehensive Care Center research staff will be secured and password protected. In this file there will be a reference key linking the participant to their unique participant ID. New credentialed personnel will be approved by IRB via a modification prior to being granted access to the study databases. At the end of the study, all study databases will be de-identified and archived at the MS Comprehensive Care Center.

14 Data Handling and Record Keeping

14.1 Data Collection and Management Responsibilities

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site PI. Imaging data will be collected at CBI at Department of Radiology of NYU Langone Health, and will be stored in the PACS system. Only the investigators of this study will have access to analyze these data. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Black ink is required to ensure clarity of reproduced copies. When making changes or corrections, cross out the original entry with a single line, and initial and date the change. DO NOT ERASE, OVERWRITE, OR USE CORRECTION FLUID OR TAPE ON THE ORIGINAL.

Copies of the electronic CRF (eCRF) will be provided for use as source documents and maintained for recording data for each participant enrolled in the study. Data reported in the eCRF derived from source documents should be consistent with the source documents or the discrepancies should be explained and captured in a progress note and maintained in the participant's official electronic study record.

Clinical data (including AEs and expected adverse reactions data) and clinical laboratory data will be entered into Redcap Database, a data capture system provided by the NYU Langone Health. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

14.2 Study Records Retention

Study documents will be retained for the longer of 3 years after close out or 5 years after final reporting/publication. These documents should be retained for a longer period, however, if required by local regulations.

14.3 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or MOP requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH E6:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the site to use continuous vigilance to identify and report deviations within 10 working days of identification of the protocol deviation, or within 10 working days of the scheduled protocol-required activity. All deviations must be addressed in study source documents, reported to NYU's IRB. Protocol deviations must be reported to the local IRB per their guidelines. The site PI/study staff is responsible for knowing and adhering to their IRB requirements. Further details about the handling of protocol deviations will be included in the MOP.

14.4 Publication and Data Sharing Policy

The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a clinical trials registration policy as a condition for publication. The ICMJE defines a clinical trial as any research project that prospectively assigns human subjects to intervention or concurrent comparison or control groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Medical interventions include drugs, surgical procedures, devices, behavioral treatments, process-of-care changes, and the like. Health outcomes include any biomedical or health-related measures obtained in patients or participants, including pharmacokinetic measures and adverse events. The ICMJE policy, and the Section 801 of the Food and Drug Administration Amendments Act of 2007, requires that all clinical trials be registered in a public trials registry such as ClinicalTrials.gov, which is sponsored by the National Library of Medicine. Other biomedical journals are considering adopting similar policies. For interventional clinical trials performed under NIH IC grants and cooperative agreements, it is the grantee's responsibility to register the trial in an acceptable registry, so the research results may be considered for publication in ICMJE member journals. The ICMJE does not review specific studies to determine whether registration is necessary; instead, the committee recommends that researchers who have questions about the need to register err on the side of registration or consult the editorial office of the journal in which they wish to publish.

FDAAA mandates that a "responsible party" (i.e., the sponsor or designated principal investigator) register and report results of certain "applicable clinical trials":

- Trials of Drugs and Biologics: Controlled, clinical investigations, other than Phase I investigations of a product subject to FDA regulation;
- Trials of Devices: Controlled trials with health outcomes of a product subject to FDA regulation (other than small feasibility studies) and pediatric post-market surveillance studies.
- NIH grantees must take specific steps to ensure compliance with NIH implementation of FDAAA.

15 Study Finances

15.1 Funding Source

This study is supported by funding from the NIH National Institute of Child Health & Human Development (R21 HD094424 – 01A1) and NYU Langone Health Multiple Sclerosis Comprehensive Care Center.

15.2 Costs to the Participant

There will be no cost to the participant during this study.

15.3 Participant Reimbursements or Payments

Participants will be paid $100 for each study visit and $50 for tDCS training visit. In summary, healthy controls will be paid $100 for their single study visit, and MS participants will be paid $350 (3 study visits and 1 tDCS training visit) for full completion of the study.

The HC subgroup will be paid $250 (baseline, tDCS training, and 1-month follow-up). HC subgroup participants who opt-in to the additional 15 minutes of imaging and tDCS will be given an additional $15.00

No additional reimbursements or payments (e.g. transportation, parking, meals etc.) will be made for the participants.

16 Study Administration

16.1 Study Leadership

The Steering Committee will govern the conduct of the study. The Steering Committee will be composed of the PI of the Coordinating Center, Dr. Leigh Charvet, and the Study Coordinators. The Steering Committee will meet in person at least annually.

17 Conflict of Interest Policy

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the trial. The study leadership has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by the NYU Langone Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor prior to participation in this study. All NYULMC investigators will follow the applicable conflict of interest policies.

18 References

1 Nitsche, M. A. *et al.* Transcranial direct current stimulation: State of the art 2008. *Brain stimulation* **1**, 206-223, doi:10.1016/j.brs.2008.06.004 (2008).
2 Antal, A. *et al.* Low intensity transcranial electric stimulation: Safety, ethical, legal regulatory and application guidelines. *Clin Neurophysiol* **128**, 1774-1809, doi:10.1016/j.clinph.2017.06.001 (2017).
3 Bikson, M. *et al.* Safety of Transcranial Direct Current Stimulation: Evidence Based Update 2016. *Brain Stimul* **9**, 641-661, doi:10.1016/j.brs.2016.06.004 (2016).
4 Tecchio, F. *et al.* Multiple sclerosis fatigue relief by bilateral somatosensory cortex neuromodulation. *Journal of neurology* **261**, 1552-1558, doi:10.1007/s00415-014-7377-9 (2014).
5 Ferrucci, R. *et al.* Transcranial direct current stimulation (tDCS) for fatigue in multiple sclerosis. *NeuroRehabilitation* **34**, 121-127, doi:10.3233/NRE-131019 (2014).
6 Kuo, M. F., Paulus, W. & Nitsche, M. A. Therapeutic effects of non-invasive brain stimulation with direct currents (tDCS) in neuropsychiatric diseases. *NeuroImage* **85 Pt 3**, 948-960, doi:10.1016/j.neuroimage.2013.05.117 (2014).
7 Kalu, U. G., Sexton, C. E., Loo, C. K. & Ebmeier, K. P. Transcranial direct current stimulation in the treatment of major depression: a meta-analysis. *Psychol Med* **42**, 1791-1800, doi:10.1017/S0033291711003059 (2012).
8 Charvet, L. E., Shaw, M. T., Haider, L., Melville, P. & Krupp, L. B. Remotely-delivered cognitive remediation in multiple sclerosis (MS): protocol and results from a pilot study. *Mult Scler J Exp Transl Clin* **1**, 2055217315609629, doi:10.1177/2055217315609629 (2015).
9 Charvet, L. *et al.* Remotely Supervised Transcranial Direct Current Stimulation Increases the Benefit of At-Home Cognitive Training in Multiple Sclerosis. *Neuromodulation*, doi:10.1111/ner.12583 (2017).
10 Glanz, B. I., Healy, B. C., Hviid, L. E., Chitnis, T. & Weiner, H. L. Cognitive deterioration in patients with early multiple sclerosis: a 5-year study. *Journal of neurology, neurosurgery, and psychiatry* **83**, 38-43, doi:10.1136/jnnp.2010.237834 (2012).
11 Mori, F. *et al.* Transcranial direct current stimulation ameliorates tactile sensory deficit in multiple sclerosis. *Brain stimulation* **6**, 654-659, doi:10.1016/j.brs.2012.10.003 (2013).
12 Lefaucheur, J. P. *et al.* Evidence-based guidelines on the therapeutic use of repetitive transcranial magnetic stimulation (rTMS). *Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology* **125**, 2150-2206, doi:10.1016/j.clinph.2014.05.021 (2014).
13 Lu, H. & Ge, Y. Quantitative evaluation of oxygenation in venous vessels using T2-Relaxation-Under-Spin-Tagging MRI. *Magnetic resonance in medicine* **60**, 357-363, doi:10.1002/mrm.21627 (2008).
14 Xu, F., Ge, Y. & Lu, H. Noninvasive quantification of whole-brain cerebral metabolic rate of oxygen (CMRO2) by MRI. *Magnetic resonance in medicine* **62**, 141-148, doi:10.1002/mrm.21994 (2009).
15 Ge, Y. *et al.* Characterizing brain oxygen metabolism in patients with multiple sclerosis with T2-relaxation-under-spin-tagging MRI. *J Cereb Blood Flow Metab* **32**, 403-412, doi:10.1038/jcbfm.2011.191 (2012).
16 Bonavita, S. *et al.* Distributed changes in default-mode resting-state connectivity in multiple sclerosis. *Multiple sclerosis* **17**, 411-422, doi:10.1177/1352458510394609 (2011).
17 Pantano, P. *et al.* Cortical motor reorganization after a single clinical attack of multiple sclerosis. *Brain : a journal of neurology* **125**, 1607-1615 (2002).
18 Rocca, M. A. *et al.* Evidence for axonal pathology and adaptive cortical reorganization in patients at presentation with clinically isolated syndromes suggestive of multiple sclerosis. *NeuroImage* **18**, 847-855 (2003).
19 Rocca, M. A. *et al.* Large-scale neuronal network dysfunction in relapsing-remitting multiple sclerosis. *Neurology* **79**, 1449-1457, doi:10.1212/WNL.0b013e31826d5f10 (2012).
20 Tona, F. *et al.* Multiple sclerosis: altered thalamic resting-state functional connectivity and its effect on cognitive function. *Radiology* **271**, 814-821, doi:10.1148/radiol.14131688 (2014).

21 Zhou, Y. *et al.* Functional homotopic changes in multiple sclerosis with resting-state functional MR imaging. *AJNR. American journal of neuroradiology* **34**, 1180-1187, doi:10.3174/ajnr.A3386 (2013).
22 Charvet, L. *et al.* in *Neuromodulation: Technology at the Neural Interface Edinburgh* (Wiley, Edinburgh, 2017).
23 Datta, A. *et al.* in *Neuromodulation: Technology Changing Lives Edinburgh* (Wiley, Edinburgh, 2017).
24 Zheng, X., Alsop, D. C. & Schlaug, G. Effects of transcranial direct current stimulation (tDCS) on human regional cerebral blood flow. *NeuroImage* **58**, 26-33, doi:10.1016/j.neuroimage.2011.06.018 (2011).
25 Meinzer, M. *et al.* Transcranial direct current stimulation and simultaneous functional magnetic resonance imaging. *Journal of visualized experiments : JoVE*, doi:10.3791/51730 (2014).
26 Datta A, D. B., Shaw M, Charvet LE, Clayton A, Gonen O, Ge Y. in *International Neuromodulation Society World Congress* 24 (Edinburgh, Scotland, United Kingdom 2017).
27 Datta A, N. K., Gonen O, Ge Y, Dobbs B, Shaw M, Charvet LE. in *International Neuromodulation Society World Congress* 13 (Edinburgh, Scotland, United Kingdom, 2017).
28 Gbadeyan, O., Steinhauser, M., McMahon, K. & Meinzer, M. Safety, Tolerability, Blinding Efficacy and Behavioural Effects of a Novel MRI-Compatible, High-Definition tDCS Set-Up. *Brain stimulation* **9**, 545-552, doi:10.1016/j.brs.2016.03.018 (2016).
29 Mintun, M. A., Raichle, M. E., Martin, W. R. & Herscovitch, P. Brain oxygen utilization measured with O-15 radiotracers and positron emission tomography. *J Nucl Med* **25**, 177-187 (1984).
30 Zhu, X. H. *et al.* Development of (17)O NMR approach for fast imaging of cerebral metabolic rate of oxygen in rat brain at high field. *Proc Natl Acad Sci U S A* **99**, 13194-13199 (2002).
31 Kety, S. S. & Schmidt, C. F. The Effects of Altered Arterial Tensions of Carbon Dioxide and Oxygen on Cerebral Blood Flow and Cerebral Oxygen Consumption of Normal Young Men. *J Clin Invest* **27**, 484-492 (1948).
32 Peng, S. L. *et al.* Optimization of phase-contrast MRI for the quantification of whole-brain cerebral blood flow. *Journal of magnetic resonance imaging : JMRI* **42**, 1126-1133, doi:10.1002/jmri.24866 (2015).
33 Loitfelder, M. *et al.* Reorganization in cognitive networks with progression of multiple sclerosis: insights from fMRI. *Neurology* **76**, 526-533, doi:10.1212/WNL.0b013e31820b75cf (2011).
34 Faivre, A. *et al.* Depletion of brain functional connectivity enhancement leads to disability progression in multiple sclerosis: A longitudinal resting-state fMRI study. *Multiple sclerosis*, doi:10.1177/1352458516628657 (2016).
35 Stagg, C. J. *et al.* Polarity-sensitive modulation of cortical neurotransmitters by transcranial stimulation. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **29**, 5202-5206, doi:10.1523/JNEUROSCI.4432-08.2009 (2009).
36 Mylius, V. *et al.* Definition of DLPFC and M1 according to anatomical landmarks for navigated brain stimulation: inter-rater reliability, accuracy, and influence of gender and age. *NeuroImage* **78**, 224-232, doi:10.1016/j.neuroimage.2013.03.061 (2013).
37 Smith, S. M. *et al.* Correspondence of the brain's functional architecture during activation and rest. *Proc Natl Acad Sci U S A* **106**, 13040-13045, doi:10.1073/pnas.0905267106 (2009).
38 Zou, Q. H. *et al.* An improved approach to detection of amplitude of low-frequency fluctuation (ALFF) for resting-state fMRI: fractional ALFF. *Journal of neuroscience methods* **172**, 137-141, doi:10.1016/j.jneumeth.2008.04.012 (2008).
39 Zuo, X. N. *et al.* Growing together and growing apart: regional and sex differences in the lifespan developmental trajectories of functional homotopy. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **30**, 15034-15043, doi:10.1523/JNEUROSCI.2612-10.2010 (2010).
40. Ayache, S.S. et al. Prefrontal tDCS decreases pain in patients with multiple sclerosis. Front Neurosci, 10 (2016), p. 147
41. Ayache, S.S. et al. Long term effects of prefrontal tDCS on multiple sclerosis fatigue: a case study. Brain Stimul, 10 (2017), pp. 1001-1002
42. Kasschau, M. et al. A Protocol for the Use of Remotely-Supervised Transcranial Direct Current Stimulation (tDCS) in Multiple Sclerosis (MS). Journal of Visualized Experiments.
43. Kasschau, M. et al. Transcranial Direct Current Stimulation is Feasible for Remotely Supervised

Home Delivery in Multiple Sclerosis. Neuromodulation: Journal of the International Neuromodulation Society 2016.
44. Vsee: World's Largest Video Telemediciine Platform. 2015.
45. Medical S: Transcranial Direct Current Stimulation.. p. Soterix Medical tDCS details.
46. Nitsche, M., & Bikson, M. (2017). Extending the parameter range for tDCS: Safety and tolerability of 4 mA stimulation. *Brain Stimul,* 10(3): 541–542
47. Khadka, N. et al. (2020). Adaptive current tDCS up to 4mA. *Brain Stimul,* 13 (2020), pp. 69-79
48. Chhatbar, P. et al. (2017). Safety and tolerability of transcranial direct current stimulation to stroke patients: a phase I current escalation study. *Brain Stimul,* 10 (2017), 553-559

19 Attachments

These documents are relevant to the protocol, but they are not considered part of the protocol. They are stored and modified separately. As such, modifications to these documents do not require protocol amendments.

Attachment A

Schedule of Events

	Healthy Controls MS Patients		MS participants and HC subgroup		MS Patients Only
Activity	**Phone Screen**	**Baseline MRI Visit**	**Daily RS-tDCS Sessions (2-20)**	**Intermediate Visit (after RS-tDCS; 1 month ± 1 week)**	**Final Visit (3 months after RS-tDCS; 4 month ± 2 weeks)**
Study team procedures					
Eligibility Screen	X			X	
Consent		X			
Documentation of Consent		X			
Medical History		X		X	
MRI Safety Questionnaire		X		X	
tDCS Tolerability Check		X		X	
Pre and Post Session Questionnaire		X	X	X	
Pain Check Questionnaire		X	X	X	X
Neuropsychological Assessment		X		X	X
Laboratory Assessments					
Pregnancy Test		X		X	
Imaging Assessments					
MRI-tDCS		X		X	