Clinical Research Protocol

Effects of non-invasive brain stimulation on cognitive function in patients with multiple sclerosis

Protocol Number:	1.0
Version Date:	Oct 1st, 2020
Investigational Product:	n/a
IND Number:	n/a
Development Phase:	Phase 1
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Coordinating Center:	If applicable

Approval:

10/1/2020

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PI or Sponsor Signature (Name and Title) *Date*

This confidential information about the research study protocol is provided for the exclusive use of investigators of this study and is subject to recall at any time. The information in this document may not be disclosed unless federal or state law or regulations require such disclosure. Subject to the foregoing, this information may be disclosed only to those persons involved in the study who have a need to know, with the obligation not to further disseminate this information.

PROTOCOL AGREEMENT

I have read the protocol specified below. In my formal capacity as Investigator, my duties include ensuring the safety of the study subjects enrolled under my supervision, as outlined in the protocol. It is understood that all information pertaining to the study will be held strictly confidential and that this confidentiality requirement applies to all study staff at this site. Furthermore, on behalf of the study staff and myself, I agree to maintain the procedures required to carry out the study in accordance with accepted GCP principles and to abide by the terms of this protocol.

Protocol Number: 1.0

Protocol Title: Effects of non-invasive brain stimulation on cognitive function in patients with multiple sclerosis

Protocol Date: 10/1/2020

Investigator Signature 10/1/2020 *Date*

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Study Background

Multiple sclerosis (MS) is a neurological disorder characterized by a wide variety of disabling symptoms including motor and sensory disturbance, cognitive deficits, visual impairment, pain, fatigue and spasticity. Notably, cognitive impairment (CI) occurs in up to 70% of people with MS (Chiaravalloti & DeLuca, 2008) and has a profound influence on a patient's personal functioning, social interaction, employment status and overall quality of life. The most commonly affected cognitive domains are attention, speed of information processing, executive function and memory (Achiron & Barak, 2003; Rogers & Panegyres, 2007), and influence everyday life, from finding an individual' telephone number in the telephone directory to making a plan for a day. Although disease modifying therapies (DMTs) that target primarily the inflammatory immunopathology of MS (Birnbaum, 2010; Lopez-Diego & Weiner, 2008) can slow progression of impairments including CI, existing DMTs are only partially effective or ineffective in *improving* impaired cognition in MS patients (Geisler et al., 1996; Lovera et al., 2010; Shaygannejad, Janghorbani, Ashtari, Zanjani, & Zakizade, 2008). *Therefore, it is of utmost importance to develop alternative therapeutic approaches to alleviate CI in MS.*

Recently, different forms of non-invasive transcranial electrical stimulation (tES) have been probed as non-pharmacological interventions in several neurological and psychiatric disorders given its safety, portability and potential for at-home application. The two main forms of non-invasive tES are: transcranial ***direct*** current stimulation (tDCS) and transcranial ***alternating*** current stimulation (tACS). Preliminary studies have shown promise that tDCS may serve as a tool to ameliorate cognition (Mattioli, Bellomi, Stampatori, Capra, & Miniussi, 2016), pain (Ayache et al., 2016; Mori et al., 2010), fatigue (Ferrucci et al., 2014; Hanken et al., 2016) and sensory deficits (Mori et al., 2013) in patients with MS. For example, Mattioli et al (2016) reported that tDCS over the prefrontal cortex improves attention, information processing speed and executive function (Mattioli et al., 2016). Furthermore, this improvement in cognition was sustained six months after the last treatment. While these studies provide intriguing support for tES as a new therapeutic strategy for MS patients, tES research in MS remains in its infancy and there are no consensus recommendations for its use in MS. *Furthermore, the effects of the tACS form of tES on cognitive function in MS patients have not yet, to our knowledge, been assessed*.

TACS applies weak sinusoidal currents to the brain through the scalp in a frequency-specific manner and is capable of entraining endogenous brain oscillations and enhancing cognitive function including attention (Hsu, Zanto, & Gazzaley, 2019; Hsu, Zanto, van Schouwenburg, & Gazzaley, 2017), working memory (Meiron & Lavidor, 2014; Polania, Nitsche, Korman, Batsikadze, & Paulus, 2012; Vosskuhl, Huster, & Herrmann, 2015), response inhibition (Joundi, Jenkinson, Brittain, Aziz, & Brown, 2012), and fluid intelligence (Pahor & Jausovec, 2014; Santarnecchi et al., 2013). TACS capitalizes on

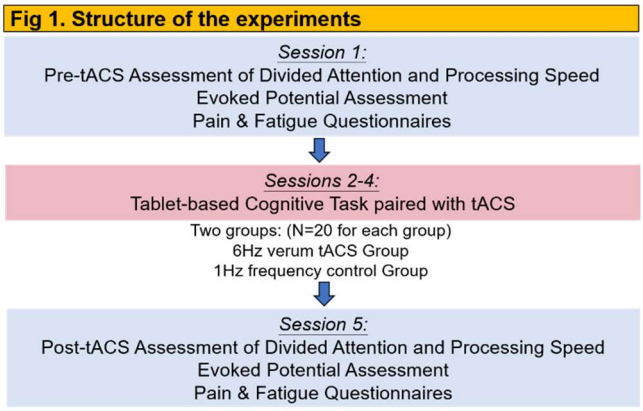
the fact that a wide range of cognitive capabilities are mediated by the dynamic modulation of rhythmic oscillatory activity, whereas tDCS only applies broad increases or decreases in cortical excitability. Therefore, tACS could provide an advantage over tDCS through a more principled approach by specifically targeting deficient oscillatory neural activity. Importantly, abnormalities in oscillatory brain dynamics have been associated with impaired cognition in MS (Kiiski et al., 2012; Van der Meer et al., 2013). For example, MS patients have significantly reduced theta power in the prefrontal cortex (PFC) compared to both healthy controls and MS patients without CI (Kiiski et al., 2012), indicating theta power reduction in the PFC is a feature of more advanced cognitive impairment in MS. Indeed, PFC theta oscillations are related to information processing tasks, especially those of attention and other executive functions (Gevins, Smith, McEvoy, & Yu, 1997; Klimesch, 1999; Mizuhara, Wang, Kobayashi, & Yamaguchi, 2004). *A collective view of these findings leads to the hypothesis that cognitive disturbance in MS may be mitigated by targeting disturbances in oscillatory brain dynamics by applying theta-tACS over the PFC.*

Study Objectives

The goals of this proposed study are to 1) evaluate the potential of using frontal theta-tACS during a cognitive task to mitigate cognitive disturbance in MS, specifically, information processing speed and attention, and 2) understand stimulation response variability at the individual-level. Processing speed and attention will be targeted for remediation due to the prevalence of their decline in MS and our previous research (and others) that show tACS may improve these cognitive functions.

Study Design

Overall strategy. We will enroll forty adult MS patients (aged 18-65 years) randomly assigned to one of two groups: either a Verum (6 Hz) tACS group or a Frequency Control (1 Hz) group. Twenty participants in each group will correspond with >80% power (1-β) at a 95% significance level (1-α) as calculated from our preliminary studies. The two groups will be age-matched. A randomized double-blinded, placebo controlled, multi-session



experiment is proposed to assess whether tACS may yield improvements in divided attention and information processing speed in MS (Specific Aim 1) and identify predictive factors of response to tACS (Specific Aim 2). The experimental paradigm consists of 5 daily sessions (conducted over up to 10 days) (Fig 1). During session 1 (baseline; pre-tACS) and session 5 (follow-up; post-tACS), participants will be assessed on divided attention while electroencephalography (EEG) data is acquired. EEG data will enable assessments of neuroplastic changes associated with tACS effects on divided attention and it will also serve to quantify cortical excitability as a predictor for tACS effects. Additionally, processing speed will be assessed along with questionnaires on pain and fatigue. During sessions 2-4, tACS will be applied while participants are engaged in a tablet-based multitasking task, which includes divided attention and processing speed components, but is distinct from the primary outcome measures.

Inclusion/Exclusion Criteria. Inclusion Criteria: age 18-65, diagnosis of MS (relapsing or progressive) according to McDonald criteria (Polman et al., 2011), Expanded Disability Status Scale (EDSS)≤6.5 (Noseworthy et al., 1990), no paresis of the upper limbs, a minimum of 3 months since the last relapse, no severe depression (Beck Depression Inventory<19), normal hearing, and no changes in MS or symptomatic medications in past 2 months. Exclusion Criteria: prior brain surgery, clips in brain, epilepsy or other neurological or non-affective psychiatric disorders, pregnancy.

Outcome measures (Sessions 1 and 5).

- **Primary outcome measure: Divided attention (Specific Aim 1).** This (and all) outcome measure(s) will be assessed at baseline (pre-tACS; session 1) and at follow-up (post-tACS; session 5). Divided attention will be measured by a divided attention task module (Table 1). The divided attention module is comprised of 3 tasks: visual perceptual discrimination (single task), visuomotor tracking (single task) and multitasking (dual task; both single tasks simultaneously). Performance during perceptual discrimination will be measured with a metric of discrimination performance (d-prime), which is estimated for each participant by comparing hit rates (correct responses to target signs) and false alarm rates (responses to non-targets) and calculated as d' = Z(hits)–Z(false alarms). D-prime cost (dual task - single

Table 1. Divided Attention Task Module

TASK	DESCRIPTION
Perceptual discrimination	Go/No Go like-paradigm; respond to target stimuli while ignoring distractors
Visuomotor tracking	Navigate a character along a dynamically moving road while avoiding walls and obstacles
Multitasking ability	Perform perceptual discrimination and visuomotor tracking tasks simultaneously

task) from the discrimination task will be used as our measure of divided attention because we have previously observed this metric to improve with tACS during this task (Hsu et al., 2019; Hsu et al., 2017) (Preliminary Study 1).

- **Primary outcome measure: Information processing speed (Specific Aim 1).** Information processing speed will be assessed with the Symbol-Digit Modalities Test (SDMT). SDMT is widely used in MS clinical trials to measure speed of information processing (Van Schependom et al., 2014). SDMT requires the participant to substitute geometric symbols for numbers while scanning a response key. Verbal, not written, responses will be recorded. The quantity of correct items will serve as the primary measure of processing speed because we have previously demonstrated that this metric improves with cognitive training in MS patients (Preliminary Study 2). Importantly, we will employ the same cognitive paradigm during tACS as we previously used in Preliminary Studies 1 and 2.
- **Secondary outcome measure: Frontal theta power (Specific Aim 1).** The visual discrimination task (single and dual task) will be used to assess neuroplastic changes in divided attention. We have previously demonstrated (and replicated) that theta-tACS above PFC is able to enhance divided attention abilities (i.e., dual - single task discrimination performance) in healthy adults (Preliminary Study 1). Importantly, increases in midline frontal theta power is correlated with improvements in divided attention. EEG data will be collected during the divided attention task module with a 64-channel BioSemi ActiveTwo system. Raw data will be segmented into epochs beginning 1000 ms before to 1000 ms post stimulus onset, demeaned/detrended and referenced to the average EEG signal. An independent component analysis will be performed to remove components consistent with topographies for blinks and eye movements. Epochs that exceed a voltage threshold of 80 μV will be rejected. EP data will focus on amplitudes and latencies of the P1 and N1 components. For the spectral analysis, a fast fourier transformation will be performed from 4 to 30 Hz. The data will be multiplied by a hanning taper, using a sliding time window. Power changes of oscillatory activity as a function of time will be calculated for theta (4-7Hz), alpha (8-12Hz), and beta (13-30Hz) bands with one power value every 10 ms.
- **Exploratory measures (Specific Aim 1).** Three exploratory measures will be used to assess whether tACS effects extend beyond our hypothesized changes in divided attention and processing speed. Specifically, during sessions 1 and 5 (pre- and post-tACS) we will assess changes in cortical excitability, fatigue and pain. 1) **Cortical excitability** will be assessed via EPs due to their sensitivity to tACS effects (Jamil et al., 2017). 2) **Fatigue** and 3) **pain** will be assessed because previous studies have shown that tDCS improves pain (Ayache et al., 2016; Mori et al., 2010) and fatigue (Ferrucci et al., 2014; Hanken et al., 2016) in patients with MS. To assess fatigue, the Modified Fatigue Impact Scale (MFIS) will be used, which consists of 21 items from the Fatigue Impact Scale (Fisk et al., 1994;

Kos et al., 2005), a multidimensional scale assessing the perceived impact of fatigue on three subscales (Physical, Cognitive, and Psychosocial) of activities, resulting in a total MFIS score. Pain will be assessed via the Brief Pain Inventory (BPI), a 32-item self-administered questionnaire to measure a pain severity score and a pain interference score (Daut, Cleeland, & Flanery, 1983).

- **Covariates to be included in Regression Analyses (Specific Aim 2).** Regression analysis will be performed to identify predictors of tACS effects on the primary outcome measures: divided attention and processing speed. Three classes of predictors will be assessed: neuroanatomy, cortical excitability, and clinical severity, in addition to those typically accounted for in MS studies: demographic (age, gender, education) and clinical features (MS duration, EDSS). 1) **Neuroanatomy**. Differences in the thickness of the skull, cerebrospinal fluid, subcutaneous fat, as well as gyral pattern, and local tissue heterogeneities (such as atrophy) yield differences in resistivity that will differentially impede current flow to cortex (Opitz, Paulus, Will, Antunes, & Thielscher, 2015; Shahid, Wen, & Ahfock, 2013; Truong, Magerowski, Blackburn, Bikson, & Alonso-Alonso, 2013). The consequence of this anatomical variability can lead to 1.5 to 3-fold differences in the induced electric field in cortex (Datta, Truong, Minhas, Parra, & Bikson, 2012; Russell et al., 2013), contributing to variable tES outcomes. Indeed, we have recently demonstrated that the amount of current that reaches the brain predicts tACS efficacy (Preliminary Study 1). Therefore, to assess the amount of tACS-induced current that reaches the brain, individual MRI data will be used to model the electric field within each patient's brain. Standard modeling software will be used for this task (Huang, Datta, Bikson, & Parra, 2019). As patients' annual clinically-acquired brain MRIs are available in Dr. Bove-led BRIDGE platform (bridge.ucsf.edu), funds are not requested to collect MRI data. The average modeled electric field magnitude within the PFC will be used as a predictor variable for the regression analysis. 2) **Cortical excitability**. Evoked potentials (EPs) from the single task version of the discrimination task will be used to assess cortical excitability as a predictor variable in the regression analysis. In MS, demyelination and axonal loss occur, which not only changes the current flow of tES, but alters cortical excitability as measured by EPs (Caramia et al., 2004). EP abnormalities in MS include morphological changes, delayed latencies, wave cancellation and increased refractory period (Iodice et al., 2016). Importantly, baseline cortical excitability, as measured by EPs, has been shown to be a significant covariate associated with variable effects of tES across individuals (Jamil et al., 2017). 3) **Clinical severity**. In a meta-analysis of non-invasive neurostimulation effects, we have shown that patients with the greatest clinical severity are the ones who benefit most from neurostimulation (Hsu, Ku, Zanto, & Gazzaley, 2015). Here, we will use the MS Functional Composite 4 (MSFC4) as a metric of clinical severity and assess it as a predictor variable in the regression analysis. The MSFC4 assesses four key MS-related functional domains: ambulation (timed 25 foot walk), upper extremity dexterity (9-

Hole Peg Test), vision (low contrast visual acuity) and cognition (PASAT, a test of information processing speed, flexibility, attention, and working memory (Rosti, Hamalainen, Koivisto, & Hokkanen, 2006; Tombaugh, 2006)).

Interventions (Sessions 2 – 4).

- **Transcranial alternating current stimulation (tACS).** TACS will be applied at 6 Hz for the Verum tACS group and 1 Hz tACS for the Frequency Control group because higher frequencies of stimulation (above theta) may introduce visual flickering (or phosphenes) that could confound the interpretation of the data (Paulus, 2010; Schutter & Hortensius, 2010). Moreover, 1-Hz tACS has not been associated with cognitive alterations (Antal et al., 2008; Castillo Saavedra et al., 2014) and we have verified its use as a control in Preliminary Study 1. A sinusoidal alternating current of 1mA peak amplitude (2mA peak-to-peak amplitude) will be delivered via a pair of Ag/AgCl electrodes (3.14 cm^2) through a Starstim-32 device (Neuroelectrics, Spain) while patients are engaged in a tablet-based cognitive task (EVO, see below). During each session, participants will receive 20 min of stimulation with a 15-s ramp up and 15-s ramp down. The stimulation electrodes will be located at F3 and F4 of the 10–20 electrode coordinate system (above PFC). Impedance will be kept below 10 kΩ. In order to confirm that the frequency control was an appropriately blinded manipulation and all patients tolerate tACS well, all patients will be asked to complete a 1-min survey to scale the perception of stimulation (headache, scalp pain, tingling, burning sensation) from 1 (mild) to 10 (severe) at the end of each session. If the score on the stimulation perception survey is below 4 for each item, tACS will be considered well-tolerated.
- **Cognitive paradigm.** During tACS, participants will be engaged in a divided attention paradigm known as EVO (Akili Labs, Boston) (Anguera et al., 2017; Anguera, Jordan, Castaneda, Gazzaley, & Arean, 2016; Arean et al., 2016; Davis, Bower, & Kollins, 2018). The EVO paradigm consists of a divided attention paradigm that combines visuomotor tracking and perceptual discrimination tasks similar to, but distinct from, those used for our outcome measure of divided attention. EVO utilizes adaptive algorithms to change game difficulty on a trial-by-trial basis for both the perceptual discrimination (adapting the response window for a target) and visuomotor tracking (adapting the speed of the forward path and sensitivity of the user's motions), with real-time feedback making the patients aware of their performance. More specifically, the adaptive algorithm makes proportional changes in gameplay difficulty when the participant's performance deviates from an 80% accuracy median, which ensures that task difficulty is equated across participants and enhances participant engagement so that the paradigm is never too easy (or boring) nor too difficult (or frustrating). Each task lasts approximately 4 minutes, with 7 task runs comprising one session (experimental day). As the

patients improve their performance throughout gameplay, they are transported to different visual "worlds" in the EVO universe, meant to immerse the participant and enhance the depth of engagement and compliance. Audio and visual cues are continuously available to the participant so they are given feedback as to their performance. Since the adaptive mechanics strive to keep the participant at ~80% accuracy, the participant is challenged to constantly improve upon their own cognitive control performance in order to reach the next level.

Using this cognitive paradigm, we have previously demonstrated that frontal theta activity is related to divided attention processes (Anguera et al., 2013). Importantly, because tACS can help entrain intrinsic neural oscillations during task performance, Preliminary Study 1 demonstrated that theta tACS during this cognitive paradigm improves divided attention abilities more so than control stimulation. Furthermore, Preliminary Study 2 has shown that a longitudinal intervention with EVO in MS patients improves processing speed. Together, these Preliminary Studies provide confidence that tACS will enhance divided attention and processing speed in MS patients through the entrainment of frontal theta oscillations that are inherent to EVO task performance.

Recruitment and Retention Plan

Recruitment. Participants will be recruited from the UCSF Center for MS and Neuroinflammation, a clinic that sees over 6,000 patients annually with MS and related disorders, with a track record of mHealth studies and trials including those led Dr. Bove. Patients will be recruited via multiple channels:

- Study investigators or staff will identify prospective subjects through chart review. For prospective participants who have been deemed eligible, coordinator will contact their clinician for permission to contact in-person at their scheduled visit, or by phone at a later date.
- Direct referral from affiliated clinicians -- study staff will follow up in person, on the phone, or over email.
- The study will be listed on ClinicalTrials.gov with contact information for responsible research staffs.

In our experience, these are generally sufficient, but if additional participants are required, Advertisements (flyers, brochures, radio or TV ads, posting on clinical research sites or social media, presentation of the study at community events/media, etc.) will be distributed. In the rare occurrence that these recruitment methods do not yield sufficient participation, we will contact previous patients from our other studies who have expressed interest in future research with us.

Retention. The proposed research requires participants to come into our lab at UCSF for 5 visits over 2 weeks. Our retention plan will focus on patient comfort and engagement. Physical comfort is achieved by placing participants in an adjustable ergonomic chair that is designed for long periods of seated activity. Additionally, breaks are offered as needed to get up, walk around, and use the restroom. Finally, water and light snacks are offered to our participants. Psychological comfort is first achieved by training all research personnel in how to be courteous and friendly with participants over email, phone and in person. Next, our research space is painted in warm and inviting colors and decorated to facilitate a relaxing experience. Finally, psychological comfort is achieved by clearly explaining the consent forms, their rights, and the tasks that the participant will be engaged in so there is no confusion or unease – and offering participants multiple opportunities to ask questions. Regarding engagement, participants are given information about the experiment that highlights the importance of this work in broader context without compromising the integrity of the experimental design. Next, the research paradigms are created to be optimally challenging, such that they are never too hard so as to be frustrating, nor are they too each so as to be boring. We believe that optimally challenging cognitive functions is the ideal way not just to keep participants engaged in the research, but will more likely yield better data. Together, our efforts to provide a comfortable and engaging research experience has historically served us well to retain participants.

Preliminary Studies

- **Preliminary study 1.** We have previously demonstrated (and replicated) that theta-tACS above PFC is able to enhance divided attention abilities in healthy young adults with a large effect size (d = 0.96) (Hsu et al., 2019; Hsu et al., 2017). More recently, we replicated these effects again in healthy older adults (aged 60 – 80 years) and further demonstrated that this improvement in attention sustains for at least a month following the last tACS treatment (manuscript in preparation). Moreover, the improvements in divided attention were correlated with increases in PFC theta activity. Although we did not assess processing speed, we did observe changes in response times during the divided attention task, suggesting processing speed may have contributed to (or co-occurred with) the improvements in divided attention. *These studies support the notion that the proposed tACS approach is a promising for treatment for cognitive decline in MS – specifically divided attention and processing speed (Specific Aim 1).* In this preliminary study, we also observed tACS effects were positively correlated with the magnitude of the individual MRI-based modeled electric field in the brain. *This provides support for our hypothesis that anatomical factors affect tACS outcomes (Specific Aim 2)*.

- **Preliminary study 2.** We have recently demonstrated the utility of the proposed experimental paradigm (i.e. EVO) and the proposed processing speed outcome measure in people with MS. Specifically, in a behavioral intervention study, MS patients who played EVO improved information processing speed (p=0.003) (Bove et al., 2019). In a follow-up randomized controlled pilot trial, the mean increase in SDMT was 6.10 for EVO task group compared with 3.55 for an active control group, with clinically improvement significantly more likely to be retained for at least two months in the EVO group (Bove et al., 2019). *These preliminary results provide additional support for the feasibility of using the proposed experimental paradigm in MS and the selection of outcome measures for Specific Aim 1.* In this preliminary study, we also demonstrated that SDMT improvement was found to be associated with clinical severity, which revealed that clinical data contributes to the change of SDMT scores. This shows that, *given the heterogeneity of MS, understanding individual variability is critical for clinical trials assessing cognitive function interventions in MS, and underscores the importance of Specific Aim 2 (Bove et al., 2019).*

Statistical Design and Power

Power. Twenty participants in a group will correspond with >80% power (1-β) at a 95% significance level (1-α) as calculated from our preliminary studies (detailed below).

Primary outcome measure: Divided attention. Results from Preliminary Study 1 showed that Verum (6 Hz) tACS, and not control tACS, yielded improved divided attention discrimination performance in healthy adults, as indexed by d-prime ($F(1,35) = 6.63$, $p = 0.01$, $\eta_p^2 = 0.16$). Here, it is hypothesized that MS patients who receive Verum tACS will exhibit comparable performance gains in divided attention. To test this, a divided attention index will be calculated from performance during the discrimination task as the cost in d-prime (i.e., d-prime dual task minus d-prime single task). Data will be submitted to an ANCOVA with Group (Verum, Control) and Session (pre-tACS, post-tACS) as factors. Although age will be matched across groups, age will serve as the covariate to account for within-group differences. A Group x Session interaction is expected to show that the Verum tACS group, compared to the control group, will exhibit a larger reduction in d-prime cost post-tACS, indicating enhanced divided attention ability. For this interaction, 20 participants per group will yield >95% power (1-β) at a 95% significance level (1-α) with an expected effect size of $f = 0.28$.

Primary outcome measure: Processing speed. Results from Preliminary Study 2 showed that cognitive training with EVO improves processing speed, as indexed by the SDMT score ($t(17) = 3.19$, $p = 0.005$, $d = 0.34$). Here, it is hypothesized that MS patients who receive Verum tACS will exhibit comparable performance gains in processing speed. To test this, SDMT score will be submitted to an ANCOVA with Group (Verum, Control) and Session (pre-tACS, post-tACS) as factors. Although age will be matched across groups, age will serve as the covariate to account for within-group differences. A Group x Session interaction is expected to show that the Verum tACS group, compared to the control group, will exhibit a higher SDMT score post-tACS, indicating enhanced processing speed. For this interaction, 20 participants per group will yield 80% power ($1-\beta$) at a 95% significance level ($1-\alpha$) with an expected effect size of $f = 0.19$.

Secondary outcome measure: Frontal theta power. Results from Preliminary Study 1 also showed that the Verum tACS group, and not control tACS, exhibited a correlation between divided attention and frontal theta power such that those participants with the greatest increase in frontal theta power yielded the greatest improvement in divided attention ability ($r = 0.50$, $p = 0.04$). Here, it is hypothesized that MS patients who receive Verum tACS will exhibit similar neuroplastic changes in frontal theta activity. To test this, frontal theta power will be extracted during the discrimination task (theta dual task minus theta single task) and a change score will be calculated (post-tACS minus pre-tACS). A regression analysis will be conducted between theta power and the change in the divided attention index (post-tACS minus pre-tACS) described above. It is expected that increases in theta power only the Verum tACS group will be related to improvements in divided attention ability. To further characterize between group differences, theta power will be submitted to an ANCOVA with Group (Verum, Control) and Session (pre-tACS, post-tACS) as factors. Although age will be matched across groups, age will serve as the covariate to account for within-group differences. A Group x Session interaction is expected to show that the Verum tACS group, compared to the control group, will exhibit a larger increase in theta power post-tACS, indicating enhanced neuroplasticity. For this interaction, 20 participants per group will yield >95% power ($1-\beta$) at a 95% significance level ($1-\alpha$) with an expected effect size of $f = 0.28$.

Dissemination Plan

Registration. PI and co-PI will ensure that the clinical trial is registered and results are submitted to ClinicalTrials.gov as outlined in the NIH policy and according to the specific timelines stated in the policy.

Informed Consent. Informed consent documents for the clinical trial will include a specific statement relating to posting of clinical trial information at ClinicalTrials.gov. Importantly, it will be clear that privacy will be maintained. Only group averages will be reported in scientific communications. Where individual data are reported (e.g., showing example representations of brain activation), individuals are not identified.

Compliance. UCSF internal policy will ensure that clinical trials are registered and results reporting occur in compliance with policy requirements.

Protection for Human Subjects

Risks To The Participants.

a. Human Participants Involvement and Characteristics: In this proposal, we will recruit people with MS. General inclusion criteria for all participants are: age 18-65, diagnosis of MS (relapsing or progressive) according to McDonald criteria, Expanded Disability Status Scale (EDSS) $\leqq$ 6.5, no paresis of the upper limbs, a minimum of 3 months since the last relapse, no severe depression (Beck Depression Inventory<19), normal hearing, and no changes in MS or symptomatic medications in past 2 months. Exclusion: prior brain surgery, clips in brain, epilepsy or other neurological or non-affective psychiatric disorders, pregnancy. Because of the difficulties in interpreting cognitive studies in participants with English as a second language, only native-English speakers will be asked to participate in the study. Participants will be selected in an unbiased fashion with regard to sex and race. Gender and minority representation issues do not interact with any of our hypotheses. Therefore, the participants that we recruit will be representative of previous populations studied at University of California, San Francisco and will be represented in the approximate proportions that we have listed. Every effort will be made to ensure that the participant population conforms to the NIH policy on Gender and Minority Inclusion in Research Study populations.

b. Sources of Materials: Behavioral and EEG data will be collected from each individual. Information obtained from the studies in the research plan will be strictly confidential, except as required by law, but will be made available to the participant in response to a specific request from the participant. All data will be identified only by a code number. There will be only one “key” linking a participant’s name and personal information with their code number. This key, and all participant information and data, is maintained in files in locked cabinets in locked lab space or password protected computer files. Only

the Principal Investigator and research staff has access to these files. Typically, only group averages are reported in scientific communications. Where individual data are reported (e.g., showing example representations of brain activation), individuals are not identified.

c. Potential Risks:

- **Electroencephalography:** There are no known risks associated with EEG procedures, all of which have been used extensively in previous research. Conventional EEG recording and sensory stimulation techniques with standard subject grounding procedures are always employed. Some electrode paste may remain on the scalp, but it can be easily removed with shampooing.
- **Transcranial alternating current stimulation:** Although uncommon, tACS has some risk for headache, dizziness, nausea, itchy sensation, irritation under the area of the electrodes and the sensation of shock. TACS has also been reported to cause brief flashes of light ("phosphenes") in humans, but these are not discomforting. Should an adverse effect occur in a participant, that participant will be excluded from continuing the intervention. The protocol described here uses stimulation levels that fall well within safety limits established by basic research investigating neural tissue damage and other possible physiological effects in both humans and animals. Overall, tACS is generally considered a safe, noninvasive and painless technique for modulating neural excitability.

Adequacy Of Protection Against Risks.

a. Recruitment and Informed Consent: Participants will be recruited from the UCSF Center for MS and Neuroinflammation, a clinic that sees over 6,000 patients annually with MS and related disorders. All participants will participate in the informed consent process. They will be familiarized with the protocol by the experimenter, including risks and benefits, and informed consent will be documented according to the regulations governing human beings as experimental participants at the University of California, San Francisco.

b. Protection Against Risks: All participants will be under close supervision throughout the experiments. All tACS, and EEG recordings are done with state of the art technology. Equipment is routinely screened to assure all components, cables etc are in perfect working condition and standard grounding techniques are always employed. All information collected from the proposed experiments will remain strictly confidential, except as required by law, and will be made available to the participant and his/her physician upon the participant’s request. Personal identification of a subject will be excluded from all scientific communications. TACS studies are closely monitored for subject comfort and tACS will be discontinued if stimulation ever feels painful. Low intensity tACS (2mA peak-to-peak

or less) will be used in the proposed studies, and research utilizing similar stimulation parameters as proposed here have reported no adverse mental or health effects lasting longer than the testing session in normal healthy subjects. Much care will be taken to ensure that stimulation parameters are within the range of published safety guidelines. Although no cases of seizures have been reported using tACS with the proposed stimulation parameters, participants will be screened for past occurrence of seizure and a family history of epilepsy as part of the inclusion/exclusion process. Participants will be fully informed of all procedures and the known and potential risks associated with tACS. Trained personnel will be present during all experimental procedures and all research staff are required to maintain current CPR certification. Drs. Bove (PI), Zanto (co-PI), Hsu (co-investigator) and Gazzaley (co-investigator) will distinguish between a serious adverse event from a non-serious adverse event and an unanticipated problem. In response to an adverse event, Dr. Bove, a board-certified neurologist at the UCSF Multiple Sclerosis Center, will monitor participants until they are either deemed fit to go home or are directed to the UCSF Medical Center for additional care. In over 33,000 sessions with over 1,000 participants using stimulation similar to the proposed protocol, here is no evidence for irreversible injury produced by transcranial electrical stimulation (tES) protocols within a wide range of stimulation parameters (≤40 min, ≤4 mA). This includes a wide variety of participants, including persons from potentially vulnerable populations. The analysis consolidates and adds to existing evidence on tES safety and facilitates further research of tES in human subjects (Bikson et al., 2016). A more recent review article suggested that safety is established for low-intensity conventional tES defined as <4mA, up to 60 min duration per day. Using tACS stimulation, fewer adverse effects were reported compared to tDCS. In specific paradigms with amplitudes of up to 10 mA, frequencies in the kHz range appear to be safe (Antal et al., 2017).

Potential Benefits Of The Proposed Research To The Participants And Others.

Participants may gain some insight into the scientific process and human cognition and, in addition, they may benefit psychologically from knowing that they are participating in important research. Participants may also experience slightly improved cognitive control abilities following tACS. The potential benefits anticipated in improved treatment and management of MS patients with cognitive decline are expected to far outweigh the minimal risks associated with these studies.

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