tDCS and Cognition in Adults With Multiple Sclerosis or Encephalitis

NCT02538094

Date: 2/28/2017

Johns Hopkins Medicine - eForm A

Transcranial Direct Current Stimulation and Cognition in Adults with Multiple Sclerosis or Encephalitis

****

1. **Abstract** (p*rovide no more than a one-page research abstract briefly stating the problem, the research hypothesis, and the importance of the research)*

Cognitive dysfunction is a common and debilitating symptom of central nervous system (CNS) neuroinflammatory disorders, including multiple sclerosis (MS) and encephalitis. Although the domains of cognition that are impaired in MS can be highly variable across individuals due to specific lesion locations, certain cognitive symptoms, such as decreased processing speed and difficulty with working memory are more universal. Numerous studies have underscored the adverse effects of cognitive dysfunction in CNS disorders on the quality of life of the affected individual. However, to date, there have been no effective treatments identified for the cognitive dysfunction seen in these populations.

The dorsolateral prefrontal cortex (DLPFC) is an attractive neuroanatomic target to address cognitive dysfunction in MS and encephalitis. This brain region has been found to play an important role in working memory and executive function, and activation of the DLPFC and its related networks during tests of working memory is clearly altered in MS. Transcranial direct current stimulation (tDCS) represents a potentially effective way of safely altering relatively localized regions of cortical functioning in a manner that may directly target the cause of faulty cerebral functioning, thereby resuscitating higher cognitive functions such as working memory. More specifically, tDCS produces a relatively localized, polarity-dependent alteration of the electrical potential in cortical tissue beneath the scalp electrode, which appears to alter the excitability of underlying cortical neurons and to modulate their firing rates. Given that DLPFC-dependent cognitive control processes are effortful and fatiguing, we anticipate that tDCS may improve cognitive functioning by reducing the "effort" needed to initiate and maintain those processes.

Our overall hypothesis is that higher cognitive functions, including working memory, can be improved by the application of anodal (stimulating) tDCS to the left prefrontal cortex. We plan to enroll two groups of individuals: 1) individuals with MS; 2) individuals with a history of encephalitis. Our primary objective will be to determine whether repeated sessions of tDCS can enhance cognitive functioning in groups; secondary objectives will include addressing cognitive fatigue and side effects of tDCS treatment. Moreover, brain MRI measures, including atrophy, lesion load and lesion location, and metabolic demand will be correlated with responsiveness to tDCS and fatigue ratings.

Overall, we anticipate that this pilot study in individuals with CNS neuroinflammatory disorders will shed light on the potential utility of tDCS to improve cognition in these populations. Moreover, this study will serve as the basis for further investigations of neuromodulation and cognitive enhancement in individuals with MS and encephalitis.

Objectives *(include all primary and secondary objectives)*
Primary Objective #1: We will investigate the utility of tDCS in improving higher cognitive functions (e.g., working memory, processing, memory, executive functioning, language functioning, learning/memory, visual perception) of individuals with multiple sclerosis and those with histories of encephalitis.

Primary Objective # 2: We will investigate the possible cumulative effect of repeated daily sessions of tDCS on cognitive functioning in individuals with multiple sclerosis and those with histories of encephalitis.

Secondary Objective #1: We will investigate the potential effects of tDCS on fatigue, both subjectively rated an as evidenced by changes in cerebral metabolic demand, in individuals with multiple sclerosis and those with histories of encephalitis.

Secondary Objective #2: We will correlate brain magnetic resonance imaging (MRI) characteristics with tDCS responsiveness and fatigue ratings.

Secondary Objective #3: We will investigate the side effects of tDCS by having participants complete a questionnaire to assess various symptoms and sensations both prior to and following stimulation.

Secondary Objective #4: We will investigate the consistency of tDCS responsiveness by having participants return for an additional session of active anodal tDCS session one month after completion of the first two (primary) study waves.

3) **Background** *(briefly describe pre-clinical and clinical data, current experience with procedures, drug or device, and any other relevant information to justify the research)*

Multiple sclerosis is a progressive disease characterized by inflammation and demyelination within the CNS. It can result in an array of symptoms including cognitive, motor, and psychiatric dysfunction, the biological bases of which are not entirely known. The nature of the disease and its seemingly idiosyncratic production of lesions throughout the brain and spinal cord result in a great deal of individual variability in both symptom profile and course. Cognitive dysfunction is a particularly common and debilitating symptom of MS (Rao 1995, Thornton and Raz 1997, Calabrese 2006). Impairments have been demonstrated in both early and late-stage disease, with prevalence rates ranging from 43 – 70% (Peyser, Rao et al. 1990, Benedict, Cookfair et al. 2006).

Numerous studies have underscored the adverse effects of cognitive dysfunction in MS on various aspects of daily life, including the ability to run a household, participate fully in society, and maintain employment, all of which negatively affect patients' overall quality of life (Chiaravalloti and DeLuca 2008, Motl, Gappmaier et al. 2011, Motl, Sandroff et al. 2011). There are currently no effective treatments for MS-related cognitive dysfunction. Studies investigating behavioral approaches to cognitive remediation and rehabilitation in MS have typically focused on improving learning and memory and have not yielded promising results (O'Brien, Chiaravalloti et al. 2008), while the prominent working memory and processing speed deficits seen in this disease remain largely unaddressed.

Following acute encephalitis, too, a variety of cognitive deficits may persist and are often the sole cause of disability. Severe cognitive deficits may be seen, and include anterograde or retrograde amnesia, aphasia, cortical disconnection syndromes, and apraxias. More typical, however, is the persistence of subtle cognitive dysfunction, including deficits in working memory, attention, reaction time, and sustained concentration (Hokkanen, Poutiainen et al. 1996, Hokkanen, Salonen et al. 1996, Hokkanen and Launes 2000, Carson, Konewko et al. 2006, Hokkanen and Launes 2007, Sejvar, Curns et al. 2008). As in MS, there are no effective treatments for cognitive dysfunction following encephalitis.

It is important to recognize that some types of cognitive impairment in MS are highly variable across individuals due to the specific location of lesions in the CNS. However, some cognitive difficulties, such as slowed processing speed (often attributed to the demyelination and disrupted transmission of neural impulses) appear more universal (e.g., Rao, Leo et al. 1991, Demaree, DeLuca et al. 1999, Benedict, Cookfair

et al. 2006). These deficits in the efficiency of cerebral processing often co-occur with deficits in other cognitive domains such as working memory (Lengenfelder, Chiaravalloti et al. 2003, DeLuca, Chelune et al. 2004) and they are hypothesized to contribute to more widespread cognitive and functional impairments in MS (Demaree, DeLuca et al. 1999). For example, processing speed and working memory impairments appear to have an interactive effect whereby exacerbations in both domains are elicited as the working memory load of a task increases (Audoin, Au Duong et al. 2005, Parmenter, Shucard et al. 2007). Thus, efforts at improving working memory capacity could also ease the deleterious effects of processing speed impairments.

Working memory reflects the brain's system for temporarily holding and manipulating information that no longer exists in the external environment (D'Esposito 2007). According Baddeley's (1986) widely studied multi-component framework, working memory is thought to function via a central executive along with a pair of "slave" systems: the phonological loop and visuospatial sketchpad. At its essence, the slave systems are proposed to be responsible for maintaining verbal and visual information, respectively, while the executive system is charged with the manipulation of this information. Revisions of the model have included the addition of an episodic buffer capable of binding multidimensional information into integrated episodes (Baddeley 2000). Early studies in MS applying Baddeley's model have demonstrated that patients experience deficits in both the storage buffer (e.g., Litvan, Grafman et al. 1988, Rao, Grafman et al. 1993) and central executive (D'Esposito, Onishi et al. 1996) aspects of working memory.

While helpful in conceptualizing the cognitive processes involved in working memory procedures, Baddeley's is by no means the only cognitive model proposed to explain working memory. For example, other models conceptualize the content of working memory not as existing within storage buffers, but instead as reflecting the fraction of data that is within one's focus of attention (Cowan 1988), or as representations that are at a high level of activation over a given (and temporary) time period (Anderson 1983). As far as we are aware, these models have not been directly tested in MS patient groups. There are also multiple subtypes of working memory (e.g. verbal, object, positional, etc.) and functional neuroimaging is beginning to provide information on the unique anatomy of these functional subtypes (see Walsh, Montojo et al. 2011's study of object working memory's reliance on the frontal-occipital fasciculus, for example). The ability of various neuromodulatory techniques to differentially alter these functional working memory regions and networks remains to be seen.

Given the lack of a single cohesive model of working memory, it is perhaps not surprising that a considerable body of research utilizing a variety of methodologies has demonstrated that working memory does not reflect a single, unitary system (Baddeley 1986, D'Esposito 2007). Despite the somewhat imprecise nature of the construct, it is nonetheless apparent that in humans the ability to temporarily hold and manipulate information is a basic cognitive function critical to the success of higher order cognitive processes such as learning and recall. Further, different forms of working memory elicited via different methods (e.g. PASAT, n-back tasks, the Sternberg paradigm) appear to be derived from a similar/adjacent neuroanatomy that has been well described in both healthy adults and those with MS.

Among normal healthy controls, studies using functional neuroimaging during a variety of working memory tasks (e.g.. using auditory, verbal and spatial stimuli) have documented relatively consistent patterns of activation that include left prefrontal and premotor frontal regions (middle and inferior frontal gyri) along with more posterior association cortices (e.g., Braver, Cohen et al. 1997, Courtney, Ungerleider et al. 1997). Patients with MS also show primarily left frontal region activation during working memory tasks, but there is also an expansion of activation to bilateral frontal and posterior areas not seen among healthy controls (Audoin, Ibarrola et al. 2003, Mainero, Caramia et al. 2004, Audoin, Au Duong et al. 2005, Chiaravalloti and DeLuca 2008). This spread of activation appears to be most evident in patients with working memory deficits. For example, in MS patients who perform as well as matched controls on working memory tasks, activation remains primarily within left frontal regions. In contrast, patients with working memory deficits activate more right frontal and parietal areas during working memory tasks (Chiaravalloti, Hillary et al. 2005). In light of the relatively well understood neuroanatomy underlying working memory, efforts aimed at improving this cognitive ability in MS may be most effective if focused on enhancing left prefrontal cortex functioning.

A rapidly growing body of evidence demonstrates that transcranial direct current stimulation (tDCS) can induce changes in physical and cognitive functioning (Stagg and Nitsche 2011), and it may represent an effective way of resuscitating higher cognitive functions such as working memory in those with MS. The technique involves passing weak direct electrical current through the scalp to produce a relatively localized, polarity-dependent alteration of the electrical potential in cortical tissue beneath the scalp electrode (Wassermann and Grafman 2005, Wagner, Valero-Cabre et al. 2007), which appears to alter the excitability of underlying cortical neurons and to modulate their firing rates, as measured by single-unit recordings in animals or evoked potential measures in animals and in humans (Priori 2003). The effects of these alterations can be excitatory (with anodal stimulation) or inhibitory (with cathodal stimulation).

As it has been previously used, tDCS is administered to the scalp, forehead, and/or upper arm (extracephalic placement) via 25-35 cm^2 saline-soaked sponges. A weak (1-2 mA) direct current is applied through the electrodes for up to 40 minutes at a time. Under these conditions, the technique has been shown to be safe (Nitsche, Liebetanz et al. 2003, Iyer, Mattu et al. 2005, Poreisz, Boros et al. 2007) and unobtrusive. Many subjects do not perceive the current being applied. Some subjects report a tingling sensation under the electrode during tDCS, although increasing the current can eliminate this perception. Depending upon the duration of stimulation, and the experimental situation, some effects of tDCS have been found to persist for minutes, hours, or up to a month (Schlaug, Hamelin et al. 2007, Mori, Codeca et al. 2010).

The prefrontal cortex, and the dorsolateral prefrontal cortex (DLPFC) in particular, is an attractive neuroanatomic target to address working memory dysfunction in MS and encephalitis via tDCS. Numerous prior investigations have demonstrated that tDCS applied to the left DLPFC can improve working memory in both healthy adults and patient groups (Ohn, Park et al. 2008, Andrews, Hoy et al. 2011). Early work by Fregni and colleagues (Fregni, Boggio et al. 2005) demonstrated the specificity of anodal stimulation to the left DLPFC in improving working memory, as indexed by an n-back task performance, in healthy adults. These results were specific to anodal stimulation of the left DLPFC, as working memory performance remained unchanged in response to both cathodal stimulation of the left DLPFC and anodal stimulation of the left primary motor cortex. Similar improvements in working memory have been seen in patients with stroke (Jo, Kim et al. 2009), Parkinson's disease (Boggio, Ferrucci et al. 2006), and major depression (Fregni, Boggio et al. 2006). Notably, in all of these investigations working memory was assessed by either verbal (letter) n-back tasks or performance on a clinically popular method of assessing working memory, the digit span task. It remains unclear whether tDCS intervention has the ability to modify other, nonverbal types of working memory. However, given 1) the size of the electrodes used, 2) the placement of electrodes over a relatively large proportion of the DLPFC, and 3) the adjacent nature of the brain regions subserving different types of working memory performance, tDCS may also be an effective means of altering nonverbal forms of working memory.

TDCS has been shown to be beneficial as well as tolerable to individuals with MS and encephalitis. For instance, Mori and colleagues (Mori, Codeca et al. 2010) demonstrated that anodal tDCS applied over the course of five days significantly lessened neuropathic pain and improved quality of life in adults with MS. Importantly, these changes persisted for up to four weeks. This same group (Mori, Nicoletti et al. 2013) has also shown that daily anodal tDCS administration in MS can improve tactile discrimination thresholds and increase sensation for up to three weeks following stimulation. Cuypers and colleagues (2013) very recently demonstrated that a single session of 1mA anodal stimulation could increase corticospinal output and projection strength in SM patients, though another significantly underpowered study by this same group failed to demonstrate improvement in motor performance (Meesen, Thijs et al. 2013). Additionally, a series of case studies reported that repeated cathodal (inhibiting) stimulation effectively reduce seizure frequency and improved alertness and language in one form of encephalitis (San-Juan, Calcáneo et al. 2011). Despite the evidence documenting the safety and utility of tDCS in MS and encephalitis, tDCS has yet to be investigated as a means of enhancing cognitive functioning in these patient populations.

Studies of tDCS provide evidence to suggest that sustained and repeated stimulation paradigms can prove effective in generating prolonged treatment effects. For example, a single 13-minute session of motor cortex stimulation has yielded up to 90 minutes of altered cortical excitability (Nitsche, Liebetanz et al. 2003), and consecutive daily sessions of tDCS were associated with a significant behavioral improvement lasting up to two weeks post-treatment in individuals experiencing post-stroke motor dysfunction (Schlaug, Hamelin et al. 2007). With respect to cognitive enhancement, it has been demonstrated that repeated daily anodal tDCS applied to the DLPFC results in improvements in working memory that last up to a week or longer in adults with major depression (Fregni, Boggio et al. 2006), and these cognitive enhancements are independent of tDCS-induced changes in mood functioning. As noted above, in MS repeated daily stimulation aimed at reducing neuropathic pain yielded significantly diminished pain ratings three weeks following the termination of stimulation and was not associated with any adverse reactions in these patients (Mori, Codeca et al. 2010). Given these results, we will provide repeated daily stimulation to our participants in order to maximize the magnitude and duration of effects that are attainable with tDCS.

The PI and Co-Investigators have extensive research and clinical experience with the study tasks and patient populations. We have been trained in the application of tDCS and have run over 130 healthy adult participants through our tDCS study paradigms. We have also investigated the potential of tDCS to alter cognition in individuals with impairments in cognition attributable to stroke, developmental disorders, and aging. The study team also regularly evaluates and treats patients with multiple sclerosis and encephalitis through our clinical practices.

4) **Study Procedures**

a. Study design, including the sequence and timing of study procedures (distinguish research procedures from those that are part of routine care).

Experimental Procedures. Patients with multiple sclerosis and those with encephalitis will be notified of the study through newspaper advertisements, public announcements, flyers at The Johns Hopkins University and Hospital and via postings at MS support groups and their associated websites. Patient groups will also be notified of the study by contacting physicians who treat patients with these conditions in outpatient clinics of the Departments of Neurology, Psychiatry, and Physical Medicine & Rehabilitation and in the community. We have a history of successful participant recruitment from these sources. We may also communicate with patients of The Johns Hopkins Hospital who, over the course of their clinical care, have previously given permission to be contacted for potential inclusion in research studies.

Participants will first be screened to determine whether they meet inclusion criteria for one of two groups:

1. Those with multiple sclerosis
2. Those with a history of encephalitis.

Group 1 - Multiple sclerosis. This group will consist of 34 adult outpatients with multiple sclerosis. Because cognitive impairment is ubiquitous across all subtypes of the disease (relapsing-remitting, primary and secondary progressive and progressive relapsing), we intend to enroll those diagnosed with any MS subtype. Such recruitment reflects the practices of other recent tDCS investigations (Ferrucci, Vergari et al. 2014, Tecchio, Cancelli et al. 2014, Tecchio, Cancelli et al. 2015) and will allow for a more thorough examination of disease subtypes and symptom parameters that affect the efficacy of the study intervention.

To determine whether patients meet study criteria and to characterize their cognitive status, they will be administered the Mini Mental State Exam, a brief cognitive screening measure (MMSE; Folstein, Folstein et al. 1975), a brief test of intellectual functioning (Hopkins Adult Reading Test; HART; Schretlen,

Winicki et al. 2009), a depression questionnaire (Beck Depression Inventory-II, BDI-II; Beck, Steer et al. 1988), and the Minimal Assessment of Cognitive Functioning in Multiple Sclerosis (MACFIMS; Benedict, Cookfair et al. 2006) or a similar assessment. They will also complete a history questionnaire to rule out any potential exclusionary factors (outlined below) and allow for the collection of data on the use of disease modifying drugs, cognitive enhancing or stimulant medications, and caffeine. Finally, they will complete a self-report measure of fatigue (Fatigue Severity Scale, FSS; Krupp, LaRocca et al. 1989) in order to assess baseline cognitive and physical symptoms of fatigue.

Group 2 – Encephalitis. A second subject group will consist of 10 adult outpatients with histories of encephalitis. To determine whether patients meet these criteria and to characterize their cognitive status, they will be administered a brief cognitive screening measure (MMSE; Folstein, Folstein et al. 1975), a brief test of intellectual functioning (HART; Schretlen, Winicki et al. 2009), the BDI-II (Beck, Steer et al. 1988), the MACFIMS (Benedict, Cookfair et al. 2006) or a similar assessment. They will also complete a history questionnaire to rule out any potential exclusionary factors (outlined below) and allow for the collection of data on the use of disease modifying drugs, cognitive enhancing or stimulant medications, and caffeine. Finally, they will complete a self-report measure of fatigue (FSS; Krupp, LaRocca et al. 1989) in order to assess baseline cognitive and physical symptoms of fatigue.

We propose to conduct a sham-controlled, cross-over experiment in which participants undergo structural and functional brain MRI studies, complete baseline cognitive screening, and engage in behavioral tasks prior to, during, and/or following the application of active and sham tDCS. Stimulation (active or sham) will take place in two waves, each lasting five to ten days over the course of one to two weeks. After a washout period of approximately four weeks, participants will repeat a second study wave wherein they engage in the same study procedures while receiving the opposite stimulation condition (active/sham). Structural and functional MRI data will be collected before and after each study wave. Based on prior studies exploring left DLPFC stimulation to enhance cognition (Fregni, Boggio et al. 2005, Boggio, Ferrucci et al. 2006, Fregni, Boggio et al. 2006, Jo, Kim et al. 2009, Andrews, Hoy et al. 2011), as well as Ohn's work (Ohn, Park et al. 2008) documenting greater effects of tDCS on working memory following longer stimulation periods and Fregni's work (Fregni, Boggio et al. 2006) documenting persistence of effects when stimulation is administered over multiple occasions, we will apply 30 minutes of 2mA anodal stimulation daily over the course of five to ten days. In order to address the issue of constancy of tDCS responsiveness, participants will return one month after the completion of study waves one and two. At this time they will receive a single session of anodal tDCS applied with the same electrode montage and stimulation parameters and will complete the same set of cognitive tests.

tDCS procedures. tDCS will be administered to relatively localized brain regions using the international 10-20 classification system to apply electrodes to the head. Specifically, to affect the left prefrontal region, the active electrode will be placed over the left prefrontal region (F3, F7 region). The indifferent (reference) electrode will be placed over either the right supraorbital region or the right dorsolateral prefrontal cortex (F4, F8 region). These patterns of electrode placement have been used successfully in numerous prior studies in the literature. As with prior studies, current will be administered via 25—100 cm^2 saline-soaked sponges. The maximal current administered through unit area will be within the guidelines established for safe administration (Nitsche, Liebetanz et al. 2003, Poreisz, Boros et al. 2007, Bikson, Datta et al. 2009).

Tasks. Participants will complete screening and characterization measures as well as a number of cognitive tasks. The latter consist of standardized neuropsychological tests and well-established tests of working memory and higher-order cognitive functioning typically used in experimental paradigms such as a computerized n-back tasks and measures of reaction time/processing speed. Participants will also complete the MACFIMS (Benedict, Cookfair et al. 2006), a 90-minute, neuropsychological battery of seven tests that was developed by an international conference of MS experts as a means of measuring the five cognitive domains commonly affected in multiple sclerosis. These include working memory, processing speed, learning and memory, executive functioning, verbal fluency, and visuoperceptual ability.

Performance on the MACFIMS as well as the other dependent measures (i.e. working memory, reaction time/processing speed) will be assessed five times for each participant: at baseline/day 1 of wave 1, at the end of the first wave, at baseline/day 1 of the wave 2, at the end of wave 2, and at the one-month follow-up.

Based on the electrode locations we chose and the cognitive deficits seen in MS, we expect to see select areas of improvement across MACFIMS subtests and other dependent measures. Specifically, we anticipate improvements in working memory and processing speed in response to active anodal stimulation relative to sham stimulation, but we do not anticipate observing any stimulation-related changes in basic visual perceptual skills, for example.

During the fMRI studies participants will perform spatial and object working memory tasks. Specifically, subjects will perform repeated trials of an object working memory task, interleaved with nonmnemonic sensorimotor control trials, for 8 minutes. Then they will perform repeated trials of a spatial working memory task for 40 minutes to induce task-specific cognitive fatigue. They will then perform 16 minutes of the same object working memory task and then 8 minutes of the spatial working memory task to measure recovery from fatigue, if any.

Participants will be blinded to tDCS condition (as discussed below) via the ramping up of stimulation over the course of several seconds. All scoring will be done offline by a dedicated study team member.

Questionnaires and screening instruments. Prior to receiving the first session of active tDCS stimulation or sham stimulation, all subjects will complete several questionnaires and brief cognitive tests to provide the information necessary to describe our sample characteristics fully and to adjust for participant characteristics in our statistical analyses.

Verbal intelligence has been demonstrated to hold moderate correlations with various cognitive abilities. To estimate verbal intelligence, participants will read aloud a list of 35 irregularly spelled words (HART; Schretlen, Winicki et al. 2009).

Other characteristics with known associations with performance on tests of higher-order cognition include illiteracy, English as a second language, educational attainment, occupation, history of learning disabilities, substance abuse and health behaviors that impact cerebral vasculature, other cerebrovascular risk factors, psychiatric and systemic illness and their treatment, traumatic brain injury, family history of several of the above-mentioned variables, and use of disease-modifying drugs and cognitively enhancing drugs such as psychostimulants or caffeine. As such, we will ask participants to complete a History Form during the course of their participation.

Participants will also complete the Edinburgh Inventory, which specifically assesses handedness (Oldfield 1971). A history of left-handedness places one at greater probability of being right hemisphere dominant for language or for being of mixed dominance. Because this investigation seeks to alter verbally-mediated cognitive abilities, knowledge of one's probability of being left hemisphere dominant for language will be an important consideration.

Participants will complete the MMSE, a brief cognitive screening measure that assesses orientation, attention, learning/memory, language functioning, and visuoconstruction skills. Administration requires approximately five minutes and will be used to ensure that all participants meet our stated inclusion criteria (i.e., non-demented as defined by a MMSE score ≥24).

Fatigue is an extremely common problem in those with MS, affecting up to a significant proportion of patients (Hadjimichael, Vollmer et al. 2008). The Fatigue Severity Scale (FSS; Krupp, LaRocca et al. 1989) or a similar brief, self-report measure will be completed by all study participants prior to and following both active anodal and sham stimulation conditions. This questionnaire will assess participants'

subjective ratings of physical and/or mental energy as well as the degree to which any lack thereof interferes with their functioning.

Depression affects up to 60% of patients with (Minden and Schiffer 1990) and can negatively impact cognitive functioning (Arnett, Barwick et al. 2008), including working memory and processing speed. Symptoms of depression will be measured via a brief self-report questionnaire such as the Beck Depression Inventory-II (Beck, Steer et al. 1988) or a similar measure prior to and following both active anodal and sham stimulation conditions.

Participants will also complete a brief questionnaire to assess physical sensations and mood experienced prior to and following each stimulation session to document the presence and severity of any tDCS-related side effects. Subjects will be asked to rate the severity of these experiences and to report any other sensations they were not asked about directly. At the end of the study each participant will also be asked whether they believed they were receiving active or sham tDCS during the various testing phases, and they will rate their degree of confidence with respect to these judgments.

Neuroimaging.

Participants will undergo structural as well as functional MRI (fMRI) protocols (resting state and activation) prior to and following each study wave. All MRI data will be acquired at the FM Kirby Research Center for Functional Brain Imaging, using a 3T Philips Achieva System and a 32-channel receive-only head coil *(Philips Healthcare, Best, The Netherlands)*.

Structural neuroimaging in MS can yield data on whole brain atrophy, cortical atrophy, and lesion volume. Greater lesion volume and atrophy (particularly subcortical) correlate with worse cognition (Rovaris and Filippi 2000, Benedict, Bruce et al. 2006). In some cases, the participant's most recent clinical brain MRI study will be sufficient, and will be obtained from their medical records. In the case that a structural clinical scan has not been obtained in the recent past, a research scan will be obtained. We chose 3T because high resolution structural images can be obtained quickly and functional imaging is also possible. We will obtain standard MRI sequences (i.e. T2, FLAIR, diffusion weighted imaging), and a 3D high resolution isotropic T1-weighted volume acquisition using a whole-brain T_2*-weighted gradient-echo, echo planar imaging (GE-EPI) pulse sequence: TR = 2000 ms; TE = 30 ms; Flip Angle = 70 degrees; SENSE Factor (AP/RL) = 2.0 (1.0/2.0); FOV (AP × FH × RL) = 200 mm × 104.5 mm × 180 mm; Number of Transverse Slices = 35 (ascending acquisition with 0.50 mm inter-slice gap); Spatial Resolution = 2.50 mm × 2.50 mm × 2.50 mm.

We will use voxel-based morphometry (VBM) methods as one approach for analyzing structural differences between groups (Giuliani, Calhoun et al. 2005). For these analyses, T1-weighted MRI images will be readied for VBM using the optimized protocol of SPM8 (Ashburner and Friston 2000, Good, Johnsrude et al. 2001). Study-specific gray and white matter templates will be created from the set of subject images to reduce spatial normalization biases. Extracted gray and white matter segments are then normalized to templates, and smoothed by convolving with a full width half maximum filter. Unmodulated images will be analyzed to identify clusters of significance. All MRI data will be rated by trained and reliable staff who are blind to clinical data.

In addition to structural neuroimaging, participants will undergo fMRI protocols before and after each wave of tDCS stimulation. Conventional MRI with VBM can be used to analyze white matter or gray matter volume, but it cannot be used to describe function. fMRI is the use of the magnetic field produced by MRI to measure the blood oxygenation level dependent (BOLD) contrast between deoxyhemoglobin (paramagnetic) and oxyhemoglobin (less paramagnetic). The amount of deoxyhemoglobin per voxel is widely assumed to decrease as local blood flow increases, and is thus thought to be a secondary measure of neural activity.

Historically, fMRI has been used to characterize neurological activity throughout the brain during specific tasks. Here, fMRI will be collected using the same scan parameters during 9 runs, lasting 8 minutes each, during performance of fatiguing object and spatial working memory tasks.

In addition to activation paradigms, it is also possible to use fMRI to characterize the functional connectivity of neuroanatomical networks by analyzing temporal correlations in BOLD signal fluctuations between discrete brain regions at rest. Functional connectivity analysis can characterize the resting-state networks of individuals or groups, and it can reveal differences in connectivity associated with atypical development, degenerative processes, and mental illness. There is evidence that transcranial direct current stimulation can be safely used to transiently alter BOLD signal associated with goal-directed activities (Baudewig, Nitsche et al. 2001, Antal, Polania et al. 2011) and the resting-state activity in functionally connected networks (e.g., Keeser, Padberg et al. 2011, Pena-Gomez, Sala-Lonch et al. 2012). Alterations in resting-state connectivity in functionally-connected networks has also been demonstrated in those with MS (Rocca, Valsasina et al. 2012, Janssen, Boster et al. 2013).

The fMRI data will be preprocessed using Matlab 7.12 (64bit, MathWorks, Inc., Natick, Massachusetts, USA) and Statistical Parametric Mapping (SPM8) software (www.fil.ion.ucl.ac.uk/spm/), with the functional connectivity toolbox (V 13i, www.nitrc.org/projects/conn/). Individual fMRI data will be preprocessed by an initial correction for timing differences between slices, realignment, spatial normalization to Montreal Neurological Institute standardized space (www.mni.mcgill.ca/), high-pass frequency filter (128 s), correction for temporal autocorrelation and spatial smoothing with a 6mm isotropic Gaussian kernel. Within the conn toolbox, motion artifacts will be explored to ensure no significant head motion occurred during acquisition, and movement parameters will be added as a first level covariate. To increase specificity for gray matter signals and to reduce impact of physiological noise such as white matter and cerebrospinal fluid signals, a bandpass filter (0.01 – 0.1 Hz) and the anatomical component based noise correction method (CompCor) will be applied using the Conn-toolbox.

b. Study duration and number of study visits required of research participants

This study will involve 11-21 study visits taking place over the course of approximately ten to twelve weeks. This includes two five-to-ten-day sessions of tDCS (involving either active or sham stimulation) and cognitive testing, as well as a four-week washout period between these sessions, and a follow-up session four weeks thereafter. Most days the study will involve 45 to 60 minutes of participation, whereas the initial and final study visits of each wave will include an additional time spent completing the consent documentation (baseline only) as well as three hours spent completing cognitive measures and neuroimaging.

c. Blinding, including justification for blinding or not blinding the trial, if applicable

Participants will be blinded to the application of active or sham tDCS. Stimulation will be delivered by a battery-driven constant current stimulator (NeuroConn DC Stimulator Plus Model 0021) or a comparable device such as the Phoresor® II Auto Model PM850, Salt Lake City, UT, or the Chattanooga Ionto. To achieve blinding, all subjects will be fitted with the tDCS electrodes placed over the appropriate stimulation sites. When using the NeuroConn device, its pre-programed "pseudo stimulation" setting will be used during sham sessions. This shamming procedure involves the automatic application of a small current pulse every 550 ms (110 μA over 15 ms) rather than constant current as is delivered in the active stimulation condition. This current pulse enables an impedance control which reliably detects any electrode disconnection. The brief duration of stimulation yields no functional effects. When using the Phoresor® II Auto Model PM850, Salt Lake City, UT, or the Chattanooga Ionto, both active and sham conditions will involve a ramping up of the current to appropriate intensity (i.e., 2 mA) over 10-15 seconds to allow subjects to habituate to the tingling sensation. At this point, the current will be ramped back down to 0 mA for individuals in the sham condition. Termination of the stimulation after the ramping up process is generally undetectable, and the brief duration of stimulation yields no functional effects.

d. Justification of why participants will not receive routine care or will have current therapy stopped

Participation in this study will not disrupt any current care or therapy.

e. Justification for inclusion of a placebo or non-treatment group

All participants will be adults with multiple sclerosis or encephalitis. Participants in both groups will undergo active and sham conditions, thus serving as their own controls.

f. Definition of treatment failure or participant removal criteria

Participants will be removed from the study if they are unable to comply with task instructions or tolerate the tDCS procedures.

g. *Description of what happens to participants receiving therapy when study ends or if a participant's participation in the study ends prematurely*

This is not a treatment study; therefore, removal from the study prior to completion should not be detrimental to the participant in any way.

5) Inclusion/Exclusion Criteria

For this study, we will assess 34 MS patients and 10 patients with encephalitis. Individuals age 18 and over will be recruited for participation. People in their 90s have received tDCS (Boggio, Khoury et al. 2009) and there is no indication that adverse events are associated in any way with increasing age. Additional inclusion/exclusion criteria are as follows:

INCLUSION CRITERIA BY STUDY GROUP	
Multiple Sclerosis	**Encephalitis**
A diagnosis of multiple sclerosis made by a physician	A diagnosis of encephalitis made by a physician

EXCLUSION CRITERIA BY STUDY GROUP	
Multiple Sclerosis	**Encephalitis**
A diagnosis of schizophrenia bipolar disorder made by a physician	A diagnosis of schizophrenia bipolar disorder made by a physician
Greater moderate or severe depressive symptoms at baseline as indicated by Beck Depression Inventory-II scores >20	Greater moderate or severe depressive symptoms at baseline as indicated by Beck Depression Inventory-II scores > 20
MMSE score of <24	MMSE score of <24
Any uncontrolled seizure disorder	Any uncontrolled seizure disorder
Any implanted metal device or hearing aids (precludes use of tDCS)	Any implanted metal device or hearing aids (precludes use of tDCS)
Use of medication shown to interact with tDCS effectiveness, including: i. Carbamazepine/Tegratol	Use of medication shown to interact with tDCS effectiveness, including: ii. Carbamazepine/Tegratol

i. Cough/cold medicines such as Dextromethorphan, Triaminic, Robitussin, Vics Formula 44, etc.	x. Cough/cold medicines such as Dextromethorphan, Triaminic, Robitussin, Vics Formula 44, etc.
ii. Flunarizine/Sibelium	xi. Flunarizine/Sibelium
iii. Propnolol/Inderal	xii. Propnolol/Inderal
iv. Sulpiride/Dogmatil	xiii. Sulpiride/Dogmatil
v. Pergolide	xiv. Pergolide
vi. Rivastigmine/Exelon	xv. Rivastigmine/Exelon
vii. Levodopa/carbidopa/levodopa	xvi. Levodopa/carbidopa/levodopa
viii. Ropinirole/Requip	vii. Ropinirole/Requip
ix. Nicotine patch	viii. Nicotine patch

6) Drugs/ Substances/ Devices

a. *The rationale for choosing the drug and dose or for choosing the device to be used*

tDCS has been established as a valid and reliable tool for at least temporarily affecting brain and behavior with minimal risks (for review, see Priori (Priori 2003)). Stimulation will be delivered by a battery-driven constant current stimulator (NeuroConn DC Stimulator Plus Model 0021) or a comparable device such as the Phoresor® II Auto Model PM850, Salt Lake City, UT, or the Chattanooga Ionto. The NeuroConn stimulator is certified as an active medical device (class IIa) by the European Union Notified Body 0118, and has been safely used in scores of published tDCS studies around the world. We reviewed the safety notes in the operator manuals provided by the manufacturers of all three devices. The stimulation parameters in our current and planned investigations do not exceed the stimulation limits or violate the safety directives specified in the operator manual. The stimulator is not connected to a mainline power source and cannot produce more than 4.5 mA of current. As stated previously, we do not propose or plan to exceed a current of 2.0 mA. We will use non-metallic, conductive rubber electrodes covered by saline-soaked sponges to minimize the potential for chemical reactions at the interface of the scalp or skin and the electrodes.

The current density, as indexed by stimulation strength (A)/electrode size, is a relevant parameter for inducing neuronal damage (Agnew and McCreery 1987). We will be altering the applied current density to determine maximum treatment efficacy while remaining within the recommended current density safety guidelines of 40 $\mu C/cm^2$ *ph (Agnew and McCreery 1987).

b. *Justification and safety information if FDA approved drugs will be administered for non-FDA approved indications or if doses or routes of administration or participant populations are changed.* N/A

c. Justification and safety information if non-FDA approved drugs without an IND will be administered. N/A

7) Study Statistics

a. *Primary outcome variable*

The primary outcome measures will be indices of cognitive function such as working memory and performance on cognitive testing (e.g. n-back, perceptual comparison test, Digit Span) under both active anodal and sham stimulation conditions. Specifically, our primary hypothesis is that anodal tDCS will result in greater offline (i.e. post-versus pre-stimulation) improvement in cognition after multiple days than does sham stimulation. Our primary outcome variable reflects the difference

between pre- and post-anodal stimulation compared to pre- and post-sham stimulation, or [(score before anodal minus score after anodal)-(score before sham minus score after sham)].

b. *Secondary outcome variables*

1. One secondary hypothesis is that anodal tDCS will result in greater online (i.e. during tDCS) improvement in cognition after multiple days than does sham stimulation. The variable reflecting this is as follows: [(score Day 1 anodal minus score Day 5 anodal)-(score Day 1 sham minus score Day 5 sham)].
2. We will investigate whether anodal tDCS results in a steeper slope of online cognitive improvement over the multiple stimulation sessions relative to sham as this will allow us to answer the question of how many days/sessions of anodal tDCS are necessary to bring about an effect on cognition that is greater than sham. The outcome variables for this analysis will include indices of cognitive function such as working memory and performance on cognitive testing (e.g. n-back, perceptual comparison test, Digit Span) as defined by means, area under the curve, and/or slopes of the trend lines fitted over the responses from each of the consecutive sessions.
3. Exploratory hypotheses will examine whether baseline cognitive functioning, level of depressive symptoms, or fatigue relate to tDCS responsivity. The outcome variables for these analyses include indices of cognitive function such as working memory and performance on cognitive testing (e.g. n-back, perceptual comparison test, Digit Span, PASAT, etc.).
4. We will investigate the potential effects of anodal tDCS on cognitive fatigue and depression relative to sham. The outcome variables for these analyses include BDI-II and FSS scores.
5. We will correlate MRI-derived brain characteristics with tDCS responsiveness and fatigue according to the methods outlined above.
6. We will investigate the side effects of tDCS by having participants complete a questionnaire to assess various symptoms prior to and following stimulation. The outcome variables for these analyses include self-reported side effects such as pain, discomfort, tingling, etc..
7. We will investigate the consistency of tDCS effects by having participants return at follow-up for a single session of anodal stimulation. The outcome variables for these analyses include indices of cognitive function such as working memory and performance on cognitive testing (i.e. n-back, perceptual comparison test, Digit Span, PASAT, etc.).

c. *Statistical plan including sample size justification and interim data analysis*

The study design and analyses used in this investigation will be a cross-over trial involving three waves of participation with each wave consisting of several consecutive days of active or sham stimulation. Each participant will be randomized to receive either active anodal or sham stimulation during the first study wave. They will complete the opposing condition at wave two. Comparison of change variables (post-pre) will be analyzed via repeated measures ANOVA and mixed models (if data permits). Variables measured online (during stimulation) over the consecutive sessions will be analyzed using two-way repeated measures ANOVA with time and stimulation as within subjects effects. If data permits, mixed models will also be fitted. Whenever the normality assumption fails, non-parametric methods will be implemented. In addition to the above analytic plan, the Biostatistics Center of The Johns Hopkins Bloomberg School of Public Health is available as a resource for statistical designs and analyses.

With respect to neuroimaging analyses, for the working memory task-related fMRI activation measures, we will first use a voxelwise general linear model to estimate beta weights for the spatial, object, and control conditions for each 8 minute task epoch. Fatigue is expected to inversely correlate with the change in activation from the beginning to the end of the 40 minute task repetition period. Higher metabolic demand at the end relative to the beginning of the repetition period is thought to reflect less

efficient (and thus fatiguing) neural processing. Significantly less activation is hypothesized to occur in response to the fatiguing WM task under the anodal stimulation condition relative to sham stimulation.

When considering the resting state MRI analyses, imaging data from each participant will be subjected to a first-level region of interest (ROI)-ROI analysis to determine the bivariate correlations between each ROI pair within three a priori-defined networks: the dorsal attention network, the executive control network, and the salience network (http://findlab.stanford.edu/functional_ROIs, Stanford University, Palo Alto, CA) (Shirer, Ryali et al. 2012). In addressing functional connectivity changes, Fisher-transformed connectivity values will be averaged across all ROI-ROI combinations within each of the three networks. Significantly greater changes in resting-state functional connectivity are hypothesized to occur under the anodal versus sham conditions and will be reflected by [(network connectivity before anodal minus connectivity after anodal)-(connectivity before sham minus connectivity after sham)].

Specific to the recruitment of the MS participants, to observe a medium effect size (difference/standard deviation = 0.5) in univariate measures of the primary outcomes, when there is no carryover effect size, a sample size of 32 (16 in each sequence of stimulation conditions) is required. This sample size was calculated by using TrialSize Package in R. From previous tDCS studies and with experience from treating MS patients, we also anticipate a dropout and exclusion rate of 5%. Therefore, to obtain the required sample size for analyses we will be recruiting up to 34 patients for this investigation.

An interim analysis of data will be performed when the number of participants who successfully completed the study is ten. All analyses proposed above will be performed and results will be reported.

d. *Early stopping rules*. N/A

8) Risks

a. Medical risks, listing all procedures, their major and minor risks and expected frequency

tDCS: It has been demonstrated that tDCS does not: (1) cause heating under the electrodes; (2) result in harmful changes on MRI; or (3) alter levels of serum neuron-specific enolase, a sensitive marker of neuronal damage (Nitsche and Paulus 2001, Nitsche, Liebetanz et al. 2003). Many subjects (up to 71%) perceive a tingling sensation under the electrode during tDCS, although "ramping up" the current can eliminate this perception. Following tDCS, the most common reported adverse effects are fatigue (35%), itching (30%), headache (12%) and nausea (3%). Fewer patients than healthy controls report such effects (Poreisz, Boros et al. 2007). Taken together, all available research suggests that prolonged application should not pose a risk of brain damage when applied according to safety guidelines. There have been rare cases of temporary skin burns related to tDCS; these have all resolved. High electrical impedance at the site of electrode contact could theoretically have been the cause of such burns. The NeuroConn Stimulator Plus monitors electrical impedance and as a safety precaution the device terminates current flow if impedance exceeds 55kΩ. The completion of the side effects questionnaire will help determine whether participants experienced any negative consequences of the stimulation and will add to the growing literature on tDCS safety and side effects.

Sham stimulations: During sham stimulation, a small current pulse every 550 ms (110 μA over 15 ms). This brief period of stimulation causes a slight itching or tingling sensation similar to that experienced during the initial period of active stimulation. During active stimulation, participants usually habituate to the physical sensations within 30-60 seconds (Gandiga, Hummel et al. 2006); which is the characteristic that is thought to allow sham stimulation to be effective without delivering enough current to modulate neural networks. Because the total current applied will remain very low we anticipate no added risk in the experimental sham conditions.

MRI: In some cases, the participant's most recent clinical structural brain MRI study will be sufficient for the current study. If an individual does not have a recent MRI scan, they will undergo a research scan as

outlined above. The risks of having an MRI are minimal. The process itself is painless. There will be no x-rays or radioactivity in the MRI. However, participants will be exposed to a high magnetic field. The magnetic field and radio waves used for MRI scans are considered too weak to do any damage to the body. There is no evidence that any harmful or adverse effects can be expected. Nothing can be proven to be absolutely safe, but the Food and Drug Administration has set guidelines for exposure to MRI studies that we will follow. There are potential side effects known from the MRI scans. MRI scanning is associated with panic attacks or distress in some individuals. Subjects may experience claustrophobia. This is fear of small enclosed places. Some people find this unpleasant. The MRI machine makes loud banging noises, so subjects will be given earplugs that will lessen the sound to prevent damage to hearing.

The MRI machine contains a strong magnet. If the subject has certain metal in or on his/her body, the magnet may move it. That could be painful and/or harmful. Metal implants may also cause burns from the radio frequency energy used in the exams.

b. Steps taken to minimize the risks

tDCS stimulation will be ramped up over the first 15-30 seconds of stimulation in order to eliminate the sensation of tingling that can occur under the electrodes during the initial moments of tDCS application.

In regards to metal implants, we will ask participants if they have any metal in their body. If there is any question regarding this, they will not undergo the neuroimaging portion of the study.

c. Plan for reporting unanticipated problems or study deviations

Adverse events will be monitored during the entire visit by the study team. The study physician will be notified immediately if any adverse events are reported. Adverse events will be monitored until they are resolved or clearly determined to be due to a subject's stable or chronic condition or intercurrent illness. Medical care will be provided, as defined in the informed consent, for any adverse event related to trial participation. Appropriate medical care will include monitoring vital signs and/or initiating transport to the Emergency Department of The Johns Hopkins Hospital for evaluation when necessary. All adverse events, regardless of intensity or causality, will are to be recorded in the study documentation and reported to the JHU IRB. Any serious adverse events will be reported to the JHU IRB within 24 hours.

d. Legal risks such as the risks that would be associated with breach of confidentiality

Participation in this study should not put participants in any legal risk, even in the case of a breach of confidentiality.

e. Financial risks to the participants

Participants will be reimbursed for their participation in this study and do not need to change any of their current medical protocols or therapy. Therefore, there is minimal financial risk to the participants.

9) Benefits

a. Description of the probable benefits for the participant and for society

We cannot ensure that this research will provide any direct, sustainable benefit to either the patients or the healthy participants who serve as control subjects. There is the possibility that participants could experience a transient improvement in aspects of cognition as a result of their participation. Patients may be supplied with a summarization of their performance on the cognitive tests administered each week.

The completion of the study could contribute to improving understanding of how the human brain monitors and controls its own cognitive functions. This study will add to the knowledge of how externally applied currents may affect the brain's operations.

10) Payment and Remuneration

a. Detail compensation for participants including possible total compensation, proposed bonus, and any proposed reductions or penalties for not completing the protocol

All participants will be reimbursed for their direct participation at the rate of $15.00 per hour or any fraction thereof. Compensation will be provided for parking. Compensation and reimbursement will be mailed by checked at the end of each session or the end of study (per the participant's request). If a subject chooses to terminate the testing session early, he/she will still be reimbursed for their participation. Participants will be involved in a study design that involves several test sessions, across a number of days. To encourage participants to finish the study, there will be an incentive of $50 at the completion of the final test session. Of course, as noted before, if they choose not to complete the whole study, they will be paid for the portion they have completed.

11) Costs

a. Detail costs of study procedure(s) or drug (s) or substance(s) to participants and identify who will pay for them

Funding for this proposed research is provided by the Science of Learning Institute, Johns Hopkins University, endowments to The Johns Hopkins University (the Therapeutic Cognitive Neuroscience Professorship and Benjamin A. Miller Family Endowment for Aging, Alzheimer's disease, and Autism), and by gifts to The Johns Hopkins University (Therapeutic Cognitive Neuroscience Research account and others).

References

Agnew, W. F. and D. B. McCreery (1987). "Considerations for safety in the use of extracranial stimulation for motor evoked potentials." Neurosurgery **20**(1): 143-147.
Anderson, J. R. (1983). The Architecture of Cognition, Harvard University Press.
Andrews, S. C., K. E. Hoy, P. G. Enticott, Z. J. Daskalakis and P. B. Fitzgerald (2011). "Improving working memory: the effect of combining cognitive activity and anodal transcranial direct current stimulation to the left dorsolateral prefrontal cortex." Brain Stimul **4**(2): 84-89.
Antal, A., R. Polania, C. Schmidt-Samoa, P. Dechent and W. Paulus (2011). "Transcranial direct current stimulation over the primary motor cortex during fMRI." Neuroimage **55**(2): 590-596.
Arnett, P. A., F. H. Barwick and J. E. Beeney (2008). "Depression in multiple sclerosis: review and theoretical proposal." J Int Neuropsychol Soc **14**(5): 691-724.
Ashburner, J. and K. J. Friston (2000). "Voxel-based morphometry - The methods." Neuroimage **11**(6): 805-821.
Audoin, B., M. V. Au Duong, J. P. Ranjeva, D. Ibarrola, I. Malikova, S. Confort-Gouny, E. Soulier, P. Viout, A. Ali-Cherif, J. Pelletier and P. J. Cozzone (2005). "Magnetic resonance study of the influence of tissue damage and cortical reorganization on PASAT performance at the earliest stage of multiple sclerosis." Hum Brain Mapp **24**(3): 216-228.
Audoin, B., D. Ibarrola, J. P. Ranjeva, S. Confort-Gouny, I. Malikova, A. Ali-Cherif, J. Pelletier and P. Cozzone (2003). "Compensatory cortical activation observed by fMRI during a cognitive task at the earliest stage of MS." Hum Brain Mapp **20**(2): 51-58.
Baddeley, A. (2000). "The episodic buffer: a new component of working memory?" Trends Cogn Sci **4**(11): 417-423.
Baddeley, A. D. (1986). Working memory. Oxford, Oxford University Press.
Baudewig, J., M. A. Nitsche, W. Paulus and J. Frahm (2001). "Regional modulation of BOLD MRI responses to human sensorimotor activation by transcranial direct current stimulation." Magn Reson Med **45**(2): 196-201.
Beck, A. T., R. A. Steer and M. G. Carbin (1988). "Psychometric properties of the Beck Depression Inventory: Twenty-five years of evaluation." Clinical Psychology Review **8**(1): 77-100.
Benedict, R. H., J. M. Bruce, M. G. Dwyer, N. Abdelrahman, S. Hussein, B. Weinstock-Guttman, N. Garg, F. Munschauer and R. Zivadinov (2006). "Neocortical atrophy, third ventricular width, and cognitive dysfunction in multiple sclerosis." Arch Neurol **63**(9): 1301-1306.
Benedict, R. H., D. Cookfair, R. Gavett, M. Gunther, F. Munschauer, N. Garg and B. Weinstock-Guttman (2006). "Validity of the minimal assessment of cognitive function in multiple sclerosis (MACFIMS)." J Int Neuropsychol Soc **12**(4): 549-558.
Bikson, M., A. Datta and M. Elwassif (2009). "Establishing safety limits for transcranial direct current stimulation." Clin Neurophysiol **120**(6): 1033-1034.
Boggio, P. S., R. Ferrucci, S. P. Rigonatti, P. Covre, M. Nitsche, A. Pascual-Leone and F. Fregni (2006). "Effects of transcranial direct current stimulation on working memory in patients with Parkinson's disease." J Neurol Sci **249**(1): 31-38.
Boggio, P. S., L. P. Khoury, D. C. Martins, O. E. Martins, E. C. de Macedo and F. Fregni (2009). "Temporal cortex direct current stimulation enhances performance on a visual recognition memory task in Alzheimer disease." J Neurol Neurosurg Psychiatry **80**(4): 444-447.
Braver, T. S., J. D. Cohen, L. E. Nystrom, J. Jonides, E. E. Smith and D. C. Noll (1997). "A parametric study of prefrontal cortex involvement in human working memory." Neuroimage **5**(1): 49-62.
Calabrese, P. (2006). "Neuropsychology of multiple sclerosis--an overview." J Neurol **253 Suppl 1**: I10-15.
Carson, P. J., P. Konewko, K. S. Wold, P. Mariani, S. Goli, P. Bergloff and R. D. Crosby (2006). "Long-term clinical and neuropsychological outcomes of West Nile virus infection." Clin Infect Dis **43**(6): 723-730.
Chiaravalloti, N., F. Hillary, J. Ricker, C. Christodoulou, A. Kalnin, W. C. Liu, J. Steffener and J. DeLuca (2005). "Cerebral activation patterns during working memory performance in multiple sclerosis using FMRI." J Clin Exp Neuropsychol **27**(1): 33-54.
Chiaravalloti, N. D. and J. DeLuca (2008). "Cognitive impairment in multiple sclerosis." Lancet Neurol **7**(12): 1139-1151.
Courtney, S. M., L. G. Ungerleider, K. Keil and J. V. Haxby (1997). "Transient and sustained activity in a distributed neural system for human working memory." Nature **386**(6625): 608-611.
Cowan, N. (1988). "Evolving conceptions of memory storage, selective attention, and their mutual constraints within the human information-processing system." Psychol Bull **104**(2): 163-191.
Cuypers, K., D. J. Leenus, B. Van Wijmeersch, H. Thijs, O. Levin, S. P. Swinnen and R. L. Meesen (2013). "Anodal tDCS increases corticospinal output and projection strength in multiple sclerosis." Neurosci Lett **554**: 151-155.
D'Esposito, M. (2007). "From cognitive to neural models of working memory." Philos Trans R Soc Lond B Biol Sci **362**(1481): 761-772.
D'Esposito, M., K. Onishi, H. Thompson, K. Robinson, C. Armstrong and M. Grossman (1996). Working memory impairments in multiple sclerosis: Evidence from a dual-task paradigm. US, American Psychological Association. **10:** 51-56.

DeLuca, J., G. J. Chelune, D. S. Tulsky, J. Lengenfelder and N. D. Chiaravalloti (2004). "Is speed of processing or working memory the primary information processing deficit in multiple sclerosis?" J Clin Exp Neuropsychol **26**(4): 550-562.
Demaree, H. A., J. DeLuca, E. A. Gaudino and B. J. Diamond (1999). "Speed of information processing as a key deficit in multiple sclerosis: implications for rehabilitation." J Neurol Neurosurg Psychiatry **67**(5): 661-663.
Ferrucci, R., M. Vergari, F. Cogiamanian, T. Bocci, M. Ciocca, E. Tomasini, M. De Riz, E. Scarpini and A. Priori (2014). "Transcranial direct current stimulation (tDCS) for fatigue in multiple sclerosis." NeuroRehabilitation **34**(1): 121-127.
Folstein, M. F., S. E. Folstein and P. R. McHugh (1975). ""Mini-mental state". A practical method for grading the cognitive state of patients for the clinician." Journal of Psychiatric Research **12**(3): 189-198.
Fregni, F., P. S. Boggio, M. C. Lima, M. J. Ferreira, T. Wagner, S. P. Rigonatti, A. W. Castro, D. R. Souza, M. Riberto, S. D. Freedman, M. A. Nitsche and A. Pascual-Leone (2006). "A sham-controlled, phase II trial of transcranial direct current stimulation for the treatment of central pain in traumatic spinal cord injury." Pain **122**(1-2): 197-209.
Fregni, F., P. S. Boggio, M. Nitsche, F. Bermpohl, A. Antal, E. Feredoes, M. A. Marcolin, S. P. Rigonatti, M. T. Silva, W. Paulus and A. Pascual-Leone (2005). "Anodal transcranial direct current stimulation of prefrontal cortex enhances working memory." Exp Brain Res **166**(1): 23-30.
Fregni, F., P. S. Boggio, M. A. Nitsche, S. P. Rigonatti and A. Pascual-Leone (2006). "Cognitive effects of repeated sessions of transcranial direct current stimulation in patients with depression." Depress Anxiety **23**(8): 482-484.
Gandiga, P. C., F. C. Hummel and L. G. Cohen (2006). "Transcranial DC stimulation (tDCS): a tool for double-blind sham-controlled clinical studies in brain stimulation." Clin Neurophysiol **117**(4): 845-850.
Giuliani, N. R., V. D. Calhoun, G. D. Pearlson, A. Francis and R. W. Buchanan (2005). "Voxel-based morphometry versus region of interest: a comparison of two methods for analyzing gray matter differences in schizophrenia." Schizophrenia Research **74**(2-3): 135-147.
Good, C. D., I. S. Johnsrude, J. Ashburner, R. N. Henson, K. J. Friston and R. S. Frackowiak (2001). "A voxel-based morphometric study of ageing in 465 normal adult human brains." Neuroimage **14**(1 Pt 1): 21-36.
Hadjimichael, O., T. Vollmer, M. Oleen-Burkey and S. North American Research Committee on Multiple (2008). "Fatigue characteristics in multiple sclerosis: the North American Research Committee on Multiple Sclerosis (NARCOMS) survey." Health Qual Life Outcomes **6**: 100.
Hokkanen, L. and J. Launes (2000). "Cognitive outcome in acute sporadic encephalitis." Neuropsychol Rev **10**(3): 151-167.
Hokkanen, L. and J. Launes (2007). "Neuropsychological sequelae of acute-onset sporadic viral encephalitis." Neuropsychol Rehabil **17**(4-5): 450-477.
Hokkanen, L., E. Poutiainen, L. Valanne, O. Salonen, M. Iivanainen and J. Launes (1996). "Cognitive impairment after acute encephalitis: comparison of herpes simplex and other aetiologies." J Neurol Neurosurg Psychiatry **61**(5): 478-484.
Hokkanen, L., O. Salonen and J. Launes (1996). "Amnesia in acute herpetic and nonherpetic encephalitis." Arch Neurol **53**(10): 972-978.
Iyer, M. B., U. Mattu, J. Grafman, M. Lomarev, S. Sato and E. M. Wassermann (2005). "Safety and cognitive effect of frontal DC brain polarization in healthy individuals." Neurology **64**(5): 872-875.
Janssen, A. L., A. Boster, B. A. Patterson, A. Abduljalil and R. S. Prakash (2013). "Resting-state functional connectivity in multiple sclerosis: An examination of group differences and individual differences." Neuropsychologia **51**(13): 2918-2929.
Jo, J. M., Y. H. Kim, M. H. Ko, S. H. Ohn, B. Joen and K. H. Lee (2009). "Enhancing the working memory of stroke patients using tDCS." Am J Phys Med Rehabil **88**(5): 404-409.
Keeser, D., F. Padberg, E. Reisinger, O. Pogarell, V. Kirsch, U. Palm, S. Karch, H. J. Moller, M. A. Nitsche and C. Mulert (2011). "Prefrontal direct current stimulation modulates resting EEG and event-related potentials in healthy subjects: a standardized low resolution tomography (sLORETA) study." Neuroimage **55**(2): 644-657.
Krupp, L. B., N. G. LaRocca, J. Muir-Nash and A. D. Steinberg (1989). "The fatigue severity scale. Application to patients with multiple sclerosis and systemic lupus erythematosus." Arch Neurol **46**(10): 1121-1123.
Lengenfelder, J., N. D. Chiaravalloti, J. H. Ricker and J. DeLuca (2003). "Deciphering components of impaired working memory in multiple sclerosis." Cogn Behav Neurol **16**(1): 28-39.
Litvan, I., J. Grafman, P. Vendrell and J. M. Martinez (1988). "Slowed information processing in multiple sclerosis." Arch Neurol **45**(3): 281-285.
Mainero, C., F. Caramia, C. Pozzilli, A. Pisani, I. Pestalozza, G. Borriello, L. Bozzao and P. Pantano (2004). "fMRI evidence of brain reorganization during attention and memory tasks in multiple sclerosis." Neuroimage **21**(3): 858-867.
Meesen, R. L., H. Thijs, D. J. Leenus and K. Cuypers (2013). "A single session of 1 mA anodal tDCS-supported motor training does not improve motor performance in patients with multiple sclerosis." Restor Neurol Neurosci.
Minden, S. L. and R. B. Schiffer (1990). "Affective disorders in multiple sclerosis. Review and recommendations for clinical research." Arch Neurol **47**(1): 98-104.
Mori, F., C. Codeca, H. Kusayanagi, F. Monteleone, F. Buttari, S. Fiore, G. Bernardi, G. Koch and D. Centonze (2010). "Effects of anodal transcranial direct current stimulation on chronic neuropathic pain in patients with multiple sclerosis." J Pain **11**(5): 436-442.

Mori, F., C. G. Nicoletti, H. Kusayanagi, C. Foti, D. A. Restivo, M. G. Marciani and D. Centonze (2013). "Transcranial direct current stimulation ameliorates tactile sensory deficit in multiple sclerosis." Brain Stimul **6**(4): 654-659.
Motl, R. W., E. Gappmaier, K. Nelson and R. H. Benedict (2011). "Physical activity and cognitive function in multiple sclerosis." J Sport Exerc Psychol **33**(5): 734-741.
Motl, R. W., B. M. Sandroff and R. H. Benedict (2011). "Cognitive dysfunction and multiple sclerosis: developing a rationale for considering the efficacy of exercise training." Mult Scler **17**(9): 1034-1040.
Nitsche, M. A., D. Liebetanz, N. Lang, A. Antal, F. Tergau and W. Paulus (2003). "Safety criteria for transcranial direct current stimulation (tDCS) in humans." Clin Neurophysiol **114**(11): 2220-2222; author reply 2222-2223.
Nitsche, M. A. and W. Paulus (2001). "Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans." Neurology **57**(10): 1899-1901.
O'Brien, A. R., N. Chiaravalloti, Y. Goverover and J. Deluca (2008). "Evidenced-based cognitive rehabilitation for persons with multiple sclerosis: a review of the literature." Arch Phys Med Rehabil **89**(4): 761-769.
Ohn, S. H., C. I. Park, W. K. Yoo, M. H. Ko, K. P. Choi, G. M. Kim, Y. T. Lee and Y. H. Kim (2008). "Time-dependent effect of transcranial direct current stimulation on the enhancement of working memory." Neuroreport **19**(1): 43-47.
Oldfield, R. C. (1971). "The assessment and analysis of handedness: the Edinburgh inventory." Neuropsychologia **9**(1): 97-113.
Parmenter, B. A., J. L. Shucard and D. W. Shucard (2007). "Information processing deficits in multiple sclerosis: a matter of complexity." J Int Neuropsychol Soc **13**(3): 417-423.
Pena-Gomez, C., R. Sala-Lonch, C. Junque, I. C. Clemente, D. Vidal, N. Bargallo, C. Falcon, J. Valls-Sole, A. Pascual-Leone and D. Bartres-Faz (2012). "Modulation of large-scale brain networks by transcranial direct current stimulation evidenced by resting-state functional MRI." Brain Stimul **5**(3): 252-263.
Peyser, J. M., S. M. Rao, N. G. LaRocca and E. Kaplan (1990). "Guidelines for neuropsychological research in multiple sclerosis." Arch Neurol **47**(1): 94-97.
Poreisz, C., K. Boros, A. Antal and W. Paulus (2007). "Safety aspects of transcranial direct current stimulation concerning healthy subjects and patients." Brain Res Bull **72**(4-6): 208-214.
Priori, A. (2003). "Brain polarization in humans: a reappraisal of an old tool for prolonged non-invasive modulation of brain excitability." Clin Neurophysiol **114**(4): 589-595.
Rao, S. M. (1995). "Neuropsychology of multiple sclerosis." Curr Opin Neurol **8**(3): 216-220.
Rao, S. M., J. Grafman, D. DiGiulio, W. Mittenberg, L. Bernardin, G. J. Leo, T. Luchetta and F. Unverzagt (1993). Memory dysfunction in multiple sclerosis: Its relation to working memory, semantic encoding, and implicit learning. US, American Psychological Association. **7:** 364-374.
Rao, S. M., G. J. Leo, L. Bernardin and F. Unverzagt (1991). "Cognitive dysfunction in multiple sclerosis. I. Frequency, patterns, and prediction." Neurology **41**(5): 685-691.
Rocca, M. A., P. Valsasina, V. Martinelli, P. Misci, A. Falini, G. Comi and M. Filippi (2012). "Large-scale neuronal network dysfunction in relapsing-remitting multiple sclerosis." Neurology **79**(14): 1449-1457.
Rovaris, M. and M. Filippi (2000). "MRI correlates of cognitive dysfunction in multiple sclerosis patients." J Neurovirol **6 Suppl 2**: S172-175.
San-Juan, D., J. e. D. Calcáneo, M. F. González-Aragón, L. Bermúdez Maldonado, A. M. Avellán, E. V. Argumosa and F. Fregni (2011). "Transcranial direct current stimulation in adolescent and adult Rasmussen's encephalitis." Epilepsy Behav **20**(1): 126-131.
Schlaug, G., S. Hamelin, A. Pascual-Leone and D. Nair (2007). Modulating brain activity to fascilitate stroke recovery. American Neurological Association, Washington DC.
Schretlen, D. J., J. M. Winicki, S. M. Meyer, S. M. Testa, G. D. Pearlson and B. Gordon (2009). "Development, psychometric properties, and validity of the Hopkins Adult Reading Test (HART)." Clinical Neuropsychologist **23**(6): 926-943.
Sejvar, J. J., A. T. Curns, L. Welburg, J. F. Jones, L. M. Lundgren, L. Capuron, J. Pape, W. C. Reeves and G. L. Campbel (2008). "Neurocognitive and functional outcomes in persons recovering from West Nile virus illness." J Neuropsychol **2**(Pt 2): 477-499.
Shirer, W. R., S. Ryali, E. Rykhlevskaia, V. Menon and M. D. Greicius (2012). "Decoding subject-driven cognitive states with whole-brain connectivity patterns." Cereb Cortex **22**(1): 158-165.
Stagg, C. J. and M. A. Nitsche (2011). "Physiological basis of transcranial direct current stimulation." Neuroscientist **17**(1): 37-53.
Tecchio, F., A. Cancelli, C. Cottone, R. Ferrucci, M. Vergari, G. Zito, P. Pasqualetti, M. M. Filippi, A. Ghazaryan, D. Lupoi, F. M. Smits, A. Giordani, S. Migliore, C. Porcaro, C. Salustri, P. M. Rossini and A. Priori (2015). "Brain Plasticity Effects of Neuromodulation Against Multiple Sclerosis Fatigue." Front Neurol **6**: 141.
Tecchio, F., A. Cancelli, C. Cottone, G. Zito, P. Pasqualetti, A. Ghazaryan, P. M. Rossini and M. M. Filippi (2014). "Multiple sclerosis fatigue relief by bilateral somatosensory cortex neuromodulation." J Neurol **261**(8): 1552-1558.
Thornton, A. E. and N. Raz (1997). "Memory impairment in multiple sclerosis: a quantitative review." Neuropsychology **11**(3): 357-366.

Wagner, T., A. Valero-Cabre and A. Pascual-Leone (2007). "Noninvasive human brain stimulation." Annu Rev Biomed Eng **9**: 527-565.
Walsh, M., C. A. Montojo, Y. S. Sheu, S. A. Marchette, D. M. Harrison, S. D. Newsome, F. Zhou, A. L. Shelton and S. M. Courtney (2011). "Object working memory performance depends on microstructure of the frontal-occipital fasciculus." Brain Connect **1**(4): 317-329.
Wassermann, E. M. and J. Grafman (2005). "Recharging cognition with DC brain polarization." Trends Cogn Sci **9**(11): 503-505.