A Randomized, Double-Blinded, Placebo Controlled Pilot Trial of the Feasibility of High Definition Transcranial Direct Current Stimulation and Cognitive Training in Patients with Mild Cognitive Impairment.

Protocol Number: 35757
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Protocol Version History

Protocol Version	Version Date	Summary of Revisions Made	Rationale
1.0	12/13/2019	Initial version	
2.0	10/30/2020	Revised Cognitive Battery, UW ADRC Recruitment letter added, Study Partner Questionnaires added to study visits, Study Partner Questionnaires description added, Study Partner compensation added, Quality of Life Questionnaires removed from study visits and secondary endpoints, exclusion criteria updated to reflect need for study partner, Study Visit Calendar updated to reflect changes	
3.0	01/13/2021	Moved follow-up MRI to Month 3, expanded visit windows, 3.1 Objectives changed, Table 3.2 updated, 6.0 and 12.1 secondary endpoints updated	Follow-up MRI moved to Month 3 as we expect long-term effects of this multifield extended approach to tDCS, expanded visit windows to account for potential COVID-19 disruptions and increased subject retention

4.0	04/15/2021	1. Increased subject recruitment to 20 subjects and study partners, 2. removed handedness from inclusion/exclusion criteria, 3. increased Day 1 visit window to 6 months, 4. removed Day 1 AE evaluation, 5. added potential for subject interchangeability between sites 6. formatting modifications	1. Increased recruitment number to account for screen fails and withdrawals 2. Revised inclusion/exclusion criteria to improve recruitment 3. Day 1 visit window extended to improve subject retention 4. Day 1 AE evaluation removed as visit occurs prior to tDCS/sham administration 5. Interchangeability between sites added to improve subject retention and dataset completion 6. Formatting modifications to improve readability
5.0	10/15/2021	1. Section 8.5 – modified to include reimbursement for hotel or other form of lodging. MCW subjects may be eligible to stay at Kathy's House	1. We are offering lodging assistance to improve enrollment and retention

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1.0 STATEMENT OF COMPLIANCE

I confirm that I have read this protocol. I will comply with the IRB-approved protocol, and applicable regulations, guidelines, laws, and institutional policies.

I agree to ensure that all staff members involved in the conduct of this study are informed about their obligations in meeting the above commitment.

Elias Granadillo, MD.

Principal investigator	Signature	Date

2.0 LIST OF ABBREVIATIONS

ADCS-PACC	Alzheimer's disease cooperative study Preclinical Alzheimer Cognitive Composite
AE	Adverse Event
CCC	Clinical Coordinating Center
CFR	Code of Federal Regulations
CRF	Case Report Form
CRO	Contract Research Organization
CT	Cognitive Training
CTCAE	Common Terminology Criteria for Adverse Events
CTMS	Clinical Trial Management Software
DCC	Data Coordinating Center
eCRF	Electronic Case Report Forms
EDC	Electronic Data Capture
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HD-tDCS	High Definition Transcranial Direct Current Stimulation
HIPAA	Health Insurance Portability and Accountability Act
IDE	Investigational Device Exemption
ICTR	Institute for Clinical and Translational Research
IRB	Institutional Review Board
MCI	Mild cognitive impairment
MOP	Manual of Procedures
NIH	National Institutes of Health
MRI	Magnetic resonance imaging
OHRP	Office for Human Research Protections
OnCore	Online Collaborative Research Environment
PHI	Protected Health Information
PI	Principal Investigator
POC	Point of Contact
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
sIRB	single IRB
SMART IRB	Streamlined, Multisite, Accelerated Resources for Trials IRB
SMP	Study Monitoring Plan
SMS	Study Monitoring Service
UP	Unanticipated Problem

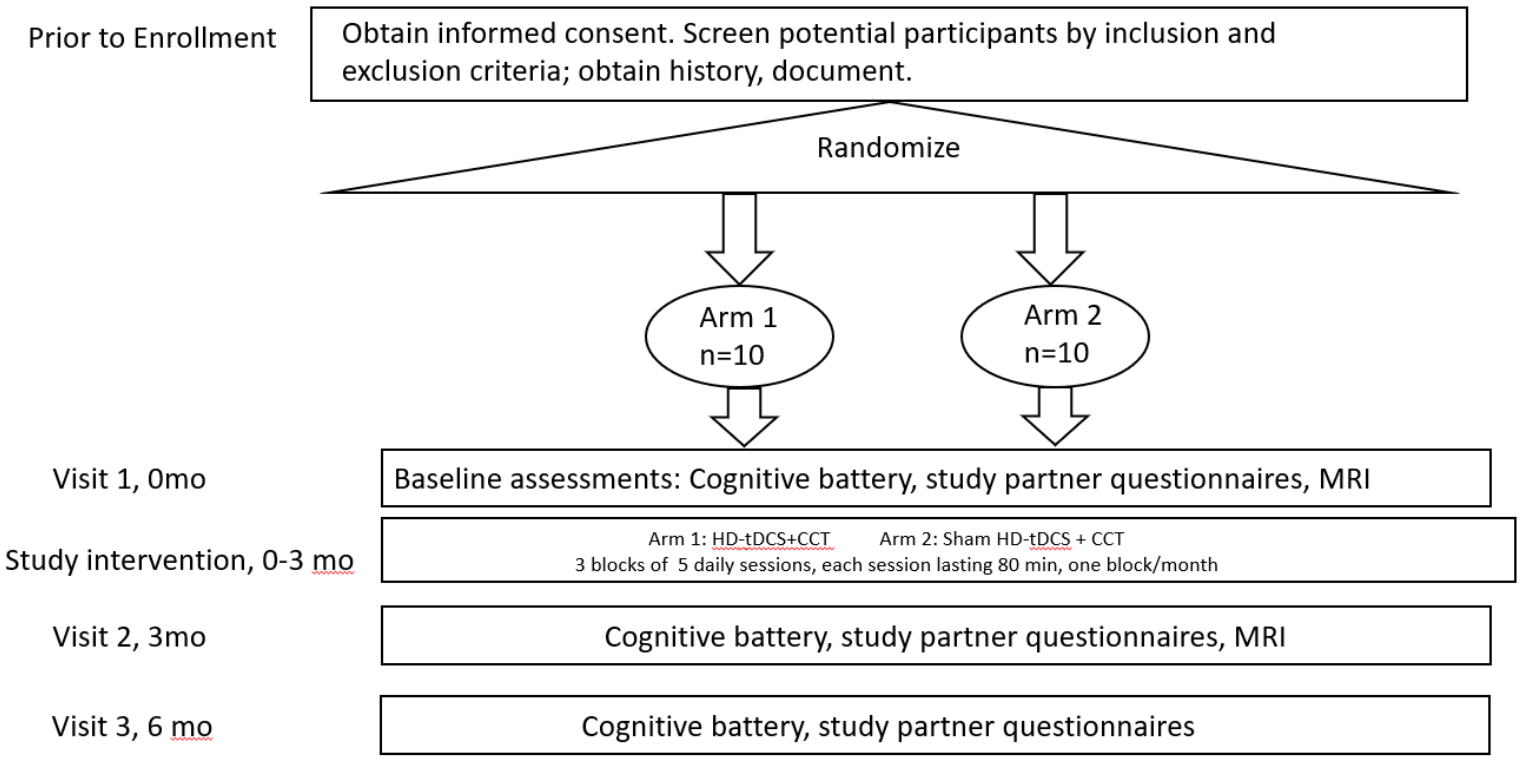
3.0 STUDY SUMMARY

3.1 Synopsis

Full Title	A Randomized, Double-Blinded, Placebo Controlled Pilot Trial of the Feasibility of High Definition Transcranial Direct Current Stimulation and Cognitive Training in Patients with Mild Cognitive Impairment.
Short Title	A pilot study of HD-tDCS and cognitive training to improve cognitive function in MCI
Protocol Number	00035757
Number of Site(s)	Two clinical sites in the United States
Phase	Pilot trial
Main Inclusion Criteria	1. Age ≥50-90 years 2. Willing and able to undergo all procedures 3. Retains decisional capacity at initial visit 4. Meets criteria for MCI, amnestic type (Petersen, 2004).
Main Exclusion Criteria	1. Significant kidney injury requiring hemodialysis 2. Automatic Internal Cardiac Defibrillator (AICD) or Pacemaker 3. Significant congestive heart failure 4. History of clinically significant ischemic or hemorrhagic stroke, or lacune or infarct considered by radiologist likely to cause or contribute significantly to cognitive symptoms 5. History of thalamic lacunar stroke 6. Modified Hachinski Ischemia Score >4 points 7. History of seizure disorder requiring medication 8. History of brain surgery (for seizure disorder, aneurysms, or benign/malignant tumor) 9. History of HIV/AIDS 10. Severe untreated obstructive sleep apnea 11. Greater than three servings alcohol daily or illicit drug use 12. Major neurologic disorders other than dementia (e.g., MS, ALS) 13. Schizophrenia, bipolar disorder, other serious mental illnesses 14. Other significant medical conditions at investigators' discretion 15. Pregnancy 16. Lack of study partner (Participants are allowed to find a new study partner if the original study partner withdraws)
Endpoints	<u>Primary Endpoint</u> • Treatment completion rate. <u>Secondary Endpoints</u>

	• Consent rate • ADCS PACC score at 3 and 6 months • Neurite density (intracellular volume fraction) at 3 months • Diffusivity indices: Fractional anisotropy (FA), Mean (MD), Axial (AD), Radial (RD) Diffusivity at 3 months • Morphometric indices: Cortical thickness, Surface Area, Volume, gyrification index at 3 months. • ASL perfusion indices: blood flow, blood volume, mean transit time, time to peak, at 3 months
Study Design	The study design is a double-blinded, randomized pilot clinical trial of HD-tDCS/sham HD-tDCS combined with CT, administered to subjects with amnestic MCI (aMCI) in blocks of 5 daily treatments for a total of 15 sessions. There will be one treatment block/month for a total of 3 months. 20 participants with aMCI will be enrolled. There will be 2 treatment groups/arms with 10 subjects per group/arm. We anticipate a dropout rate of appr. 10% and aim to complete procedures in 20 subjects.
Study Intervention	High definition transcranial direct current stimulation (HD-tDCS) Sham HD-tDCS Computerized Cognitive training (CCT) Sham CT
Total Number of Subjects	20
Study Population	Male and females aged 50 to 90 years with established diagnosis of MCI amnestic type. Total of 20 subjects
Statistical Methodology	Given the pilot nature of this study, two feasibility outcomes will be included: Consent rate and treatment completion rate. 20 patients are enough to calculate a 90% exact binomial confidence interval of (.75, 1) if all 20 are observed to complete treatment. Thus, this sample size is large enough to potentially exclude completion rates of 3/4 or lower. The pilot data from this study will provide initial estimates of the variability and the effect sizes. This information will then be used for the formal calculations of power and sample size necessary to conduct a future phase II trial.
Estimated Subject Duration	The duration of the study for each subject is approximately 6 months.
Estimated Enrollment Period & Study Duration	Study enrollment and follow-up will occur over 9 months with the total expected duration of the trial to be 12 months.

Schematic of Study Design



4.0 KEY ROLES

The following is a list of all key personnel and roles:

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5.0 INTRODUCTION

5.1 Mild Cognitive Impairment

Mild cognitive impairment (MCI) is currently an area of considerable clinical and research interest because of the high rate of conversion from MCI to Alzheimer disease (AD) (Almkvist et al., 1998; Collie et al., 2000; Kluger et al. 1999; Morris et al., 2001; Petersen et al., 1999; 2001; Rubin et al., 1989; Shah et al., 2000; Wolf et al., 1998). Several studies have demonstrated that patients with MCI progress to AD at a higher rate (10 to 15% per year) than normal elderly patients (1 to 3% per year). Therefore, MCI patients are considered to be at a higher risk for AD. MCI may be classified as either amnestic or non-amnestic, with the primary clinical distinction being the presence of prominent memory impairment in the former, and the predominant involvement of cognitive domains different from memory in the latter. Accordingly, amnestic MCI likely represents a prodromal stage of Alzheimer's dementia (Petersen et al., 2001); it is distinguished by impairment of episodic memory, and when contrasted with normal aging amnestic MCI is associated with a high degree of memory impairment with little or no impairment in activities of daily living (ADLs) (de Leon et al., 1993; Petersen et al., 2001). Individuals with amnestic MCI do not meet the currently accepted clinical criteria for probable AD, but the high rate of conversion from amnestic MCI to AD makes early diagnosis and treatment an important clinical issue. In its earliest stages, AD manifests primarily as cognitive impairment; As AD progresses, there is further loss of cognitive abilities, a loss of functional independence, and the development of behavioral problems (Gauthier, 1998). Early diagnosis and treatment may delay the onset of these symptoms.

5.2 Current Standard of Care

Currently, no drug has proven effective in treatment of MCI. Cholinesterase inhibitors have not been shown to decrease risk of progression from MCI to dementia at 1 and 3 years (Lin et al., 2013; Petersen et al., 2005; Salloway et al., 2004). In addition, cholinesterase inhibitors have limited or no significant effects on cognitive function over the short term (<12 months) and may substantially increase adverse effects, based on a meta-analysis of 4 trials (1,960 participants) (Lin et al., 2013) and another meta-analysis of 9 trials (5,149 participants) (Russ et al., 2012). Consequently, cholinesterase inhibitors and memantine are not recommended for MCI treatment and there are currently no FDA-approved medications for MCI (Lin et al., 2013; Russ et al., 2012). Ginkgo biloba, a widely used herbal supplement to improve cognition and memory, has not been shown in randomized trials to prevent cognitive decline in those with MCI or normal cognition (Snitz et al., 2009). Similarly, testosterone supplementation in older men showed no benefit for cognitive function in a randomized controlled trial (Emmelot-Vonk et al., 2008).

From 1998 to 2019 there have been about 150 failed attempts at developing Alzheimer's drugs, including medications for the management of MCI. The 'Amyloid Hypothesis' has been the leading scientific framework for the development of AD cures, and despite recent setbacks, a countless number of biologically specific AD treatments are currently being tested in clinical trials and in the drug development pipeline (Cummings et al., 2018). The recent high-profile failures highlight the importance of developing therapies that go beyond the targeting of amyloid and tau in order to help restore alternative physio-pathologic mechanisms; this would include changes in metabolism, innate immunity, modifiable lifestyle factors, and abnormal patterns of brain network connectivity and plasticity.

The restoration of brain network connectivity and of the brain's intrinsic plastic properties seems particularly relevant considering recent findings suggesting that adult hippocampal neurogenesis is active in adult healthy subjects and drops markedly in patients with AD (Moreno-Jimenez et al., 2019; Boldrini et al., 2018).

5.3 Transcranial Direct Current Stimulation and cognitive training

5.3.1. Transcranial direct current stimulation (tDCS) is a method which enables noninvasive electrical stimulation of the cortex via electrodes placed on the subject's head (Paulus, 2011; Schlaug and Renja, 2008), Anodal stimulation facilitates, and cathodal stimulation inhibits spontaneous neuronal activity in the underlying cortical areas (Nitsche et al., 2007; 2008; Priori et al., 2009). tDCS does not induce neuronal firing, but modulates spontaneous neuronal network activity (Bindman et al., 1964a; Nitsche and Paulus, 2000; Purpura and McMurty, 1965). Changes in excitability are reflected in spontaneous firing rates and responsiveness to afferent synaptic inputs (Bindman et al., 1964b; Creutzfeldt et al., 1962).
tDCS also elicits a variety of after-effects; it modifies the synaptic microenvironment, modifies synaptic strength in an NMDA-receptor dependent fashion (similar to long-term potentiation), modulates intracortical and corticospinal neurons, promotes neurogenesis (Pikhovych et al., 2016; Braun et al., 2016) and may cause transient changes in the density of protein channels in neuronal membranes (Liebetanz et al., 2002; Nitsche et al., 2003; Stagg et al., 2009). An interesting after-effect of tDCS is modulation of spontaneous neuronal oscillations (Ardolino et al., 2005). Remarkable is also the fact that constant electrical fields influence vessels and connective tissue, inflammation, cell migration, vascular motility and cellular structures (Merzagova et al., 2010).

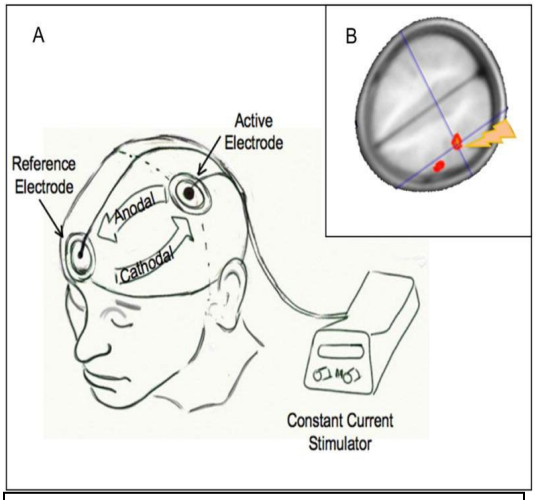


Figure 1. **TDCS setup and montage** A) TDCS setup using a mobile, battery-operated direct current stimulator connected with two electrodes. One electrode (active) is positioned over C3 (corresponding to the precentral gyrus) and the reference electrode is positioned over the contralateral supraorbital region. If current flows from C3 to the supraorbital region, then the tissue underlying C3 is subjected to anodal (increase in excitability) stimulation. If current is reversed, then the tissue underlying C3 is subjected to cathodal (decrease in excitability) stimulation; B) Regional cerebral blood flow increases in the motor region underlying the electrode 7 positioned over C3 after anodal stimulation. Regional cerebral blood flow was determined using a non-invasive arterial spin-labeling technique (adopted from Paulus, 2011).

In the 1960s, Bindman (et al., 1964b) showed that potential gradients produced by currents of the order of 0.1-0.5 mA produced neuronal excitability shifts in rat cortex and could last for many hours after the current was switched off. Recently, modern TMS techniques allowed to prove that tDCS in humans can modulate cortical excitability caused by TMS. Cathodal polarization reduced the size of the TMS-induced motor evoked potentials (MEPs), indicating reduced cortical excitability, while anodal stimulation increased the size of the MEPs, suggesting increased excitability (Paulus, 2011). The effects outlasted the duration of the stimulation. Improved performance after tDCS has been shown in motor learning, visuomotor coordination and probabilistic classification in humans (Paulus, 2011, Schlaug and Renja, 2008). Sinusoidally applied tACS allows manipulation of intrinsic cortical oscillations with externally applied electrical frequencies. Combination of tACS and tDCS has been shown to boost memory (Paulus, 2011).

Our understanding of the mechanisms of tDCS and tACS is emerging. tDCS is said to provide a subthreshold stimulus which modulates the likelihood that neurons will fire by hyperpolarizing or depolarizing brain tissue. The fact that the effects outlast current stimulation suggests that LTP and LTD are modulated (Stagg et al., 2018).

tDCS has been used to modify and study cognitive functions in healthy humans and in patients with neuropsychiatric conditions. Anodal and cathodal tDCS modulate visual working memory but can also disrupt practice-dependent improvement during a verbal working memory task when the cerebellum is stimulated (Ferrucci et al., 2008). Anodal tDCS to the anterior temporal lobes influences memories, improves decision making, attention, learning, language and memory consolidation, improves motor function in stroke patients, and improves depressive symptoms (Barham et al., 2016; Boggio et al., 2007; Brunoni et al., 2012; Cappon et al., 2000; Ferrucci et al., 2008; Fregni et al., 2006; 2007; 2015; Mattai et al., 2011; Paulus, 2011; Varga et al., 2011; Young et al., 2013). However, all these phenomena are transient. The effects of repeated applications of tDCS and the potential of this technique to lead to lasting neurocognitive improvements remain unexplored.

5.3.2. Cognitive Training

Cognitive decline is associated with risk for functional decline, nursing home placement, and mortality (Sands et al., 2002; Yaffe et al., 2002; 2006). In older individuals, concerns about forgetfulness are widespread and are associated with depression and anxiety (Reese et al., 1999; Zelinski et al., 2004; Mol et al., 2007). Interventions that reliably improve cognitive function thus have the opportunity to substantially improve the health and quality of life of older individuals. There is also literature suggesting that cognitive training (CT) can improve cognitive performance in older adults, who are at risk for dementia and MCI. Reports state that CT may significantly reduce the risk of dementia (Edwards et al., 2017). There are encouraging studies which show that CT can slow cognitive decline in individuals with MCI (Lin et al., 2016) and improve memory in older adults (Mahncke et al., 2006; Smith et al., 2009).

In response to this, two general approaches for maintaining or improving cognitive function in older adults emerged. The first focuses on direct instruction of putatively useful strategies (Naveh-Benjamin et al., 2007; Derwinger et al., 2005; McDougall, 1999; Rebok and Balcerak 1989; Willis and Nesselroade 1999; O'Hara et al., 2007). Although improvement on cognitive tests is generally seen after direct strategy instruction, performance gains typically do not generalize beyond tasks corresponding directly to the strategies taught (Verhaeghen et al., 1992; Fillit et al., 2002; Rebok et al., 2007), and it is not clear that older adults continue to use learned strategies over time (Rebok et al., 2007). As a result, strategy training programs have not been widely adopted. A second approach is derived from studies in animals (van Praag et al., 2000) and humans (Scarmeas et al., 2001; Wilson et al., 2007; Verghese et al., 2003) which suggest that nonspecific cognitive stimulation reduces the risk of cognitive decline. This has led to the practice of encouraging older adults to engage in everyday cognitively stimulating activities (Fillit et al., 2002; Small 2002; Hultsch et al., 1999), but the retrospective and observational designs of the human studies have led to difficulty interpreting the direction of causation between cognitive function and cognitively stimulating activities (Hultsch et al., 1999).

Recognition of the importance of sensory system function to cognitive function has prompted the development of a novel approach for treating age-related cognitive decline. It has been proposed that age-related reductions in the quality of neural information flowing through peripheral and central sensory systems to cognitive systems contribute to age-related cognitive decline (Schneider et al., 2000; Wingfield and Stine-Morrow 2000). Animal and human studies have demonstrated that the performance of sensory systems in the cerebral cortex can be substantially improved through intensive learning and practice and that plastic brain changes across networks of relevant cortical

areas in the central nervous system mediate these improvements (Gilbert et al., 2001; Buonomano and Merzenich 2001). Consequently, a cognitive training program designed to improve central sensory system function could potentially improve cognitive function in older adults (Mahncke et al., 2006).

Researchers utilized a computer-based cognitive training program (Brain Fitness Program, Posit Science, San Francisco, CA) in a large randomized controlled two-arm clinical trial called the Improvement in Memory with Plasticity-based Adaptive Cognitive Training (IMPACT) study (Smith et al., 2009). This program was designed to provide computer-based intervention to improve the function of brain systems through intensive brain plasticity–based learning and had shown promise in smaller-scale studies (Mahncke et al., 2006a; 2006b).

Their primary objective was to evaluate the efficacy of their experimental treatment (ET) training program by comparing the magnitude of improvements on untrained measures of memory and attention between the ET training program and an active control (AC) training program that engaged learning processes but was not designed to improve sensory function. Participants were community-dwelling adults aged 65 and older without a diagnosis of cognitive impairment. They were randomized to receive the ET or a novelty- and intensity- matched general cognitive stimulation program modeling treatment (AC). Duration of training was 1 hour per day, 5 days per week, for 8 weeks, for a total of 40 hours. Results revealed that the ET group demonstrated improvement on neuropsychological measures of memory and attention when compared to AC. Multiple secondary measures of memory and attention showed significantly greater improvements in the ET group (word list total score, word list delayed recall, digits backwards, letter–number sequencing), as did a participant-reported outcome measure. However, no advantage for the ET group was seen on a measure of narrative memory. This computer-based cognitive training program has since been successfully applied in various populations, including MCI, healthy aging, multiple sclerosis, ADHD, schizophrenia, and cardiac disease. It is now called Posit Science Brain HQ.

5.4 Rationale

Here we propose to study the feasibility and the combined effect of HD-tDCS and CT, on cognitive and imaging biomarkers of AD in subjects with aMCI. With CT we aim to activate neuronal networks within selected brain regions by engaging participants in suitably chosen tasks, while simultaneously boosting synaptic activity and plasticity of activated networks by targeting them with HD-tDCS. We will use a self-developed and safety tested HD-tDCS protocol (Turski et al., 2017), sequentially stimulate four brain regions while participants simultaneously perform appropriately chosen CT tasks that aim to engage networks within those regions. Multifocal stimulation is key to our approach, as, in contrast to previous work, we aim to improve multiple components of cognition. We will use sequential stimulation of **multiple cortical areas** over **3 months** and will **test sustainability** of achieved effects on cognition at 6 months. We hypothesize that the proposed combined treatment will improve cognition in MCI, the effect will be sustainable, and the combination will achieve superior effects compared to CT alone. In addition, we will evaluate impact of treatment on **quality of life, structural markers of neuronal connectivity, and evolution of neurodegeneration**. We are hopeful that this pilot trial will generate valuable knowledge to help design a large-scale phase II clinical trial to further explore whether the combination of HD-tDCS and CT in MCI can prevent progression to dementia.

The overall rationale for HD-tDCS (as opposed to tDCS) is straightforward: tDCS produces low-intensity electric fields across significant sections of the brain, and, while these intensities and tDCS in general are considered well tolerated and safe, the stimulation is not targeted to any particular brain region. HD-tDCS aims to produce the same low-intensity electric field but in a limited region of the brain (Figure 2). By producing the same intensity electric field in the brain but over a reduced area, HD-tDCS may add an additional safety factor to tDCS. HD-tDCS also uses electrodes designed and validated specifically for DC stimulation – again while conventional tDCS with sponge-pad based approaches is considered well tolerated, this provides an additional safety factor. Generally, the present clinical paradigm for tDCS uses two relatively large electrodes to inject current through the head resulting in electric fields that are broadly distributed over large regions of the brain. Datta et al (2009) proposed a simple method to restrict the spread of current flow using a 4x1 configuration (Figure 2). Minhas et al. (2010) showed that optimized HD-electrodes provide control over sensation and voltage. Dmochowski et al (2011) presented a method that uses multiple small electrodes (i.e. 1.2 cm diameter) and systematically optimizes the applied currents to achieve effective and targeted stimulation while ensuring safety of stimulation. They described a fundamental trade-off between achievable *intensity* (at the target) and *focality*, and algorithms to optimize both measures. When compared with large pad-electrodes (approximated here by a set of small electrodes covering 25 cm^2), the proposed approach achieves electric fields, which exhibit simultaneously greater focality (80% improvement) and higher target intensity (98% improvement) at cortical targets using the same total current applied. These improvements illustrate the previously unrecognized and non-trivial dependence of the optimal electrode configuration on the desired electric field orientation and the maximum total current (due to safety). Similarly, by exploiting idiosyncratic details of brain anatomy, the optimization approach

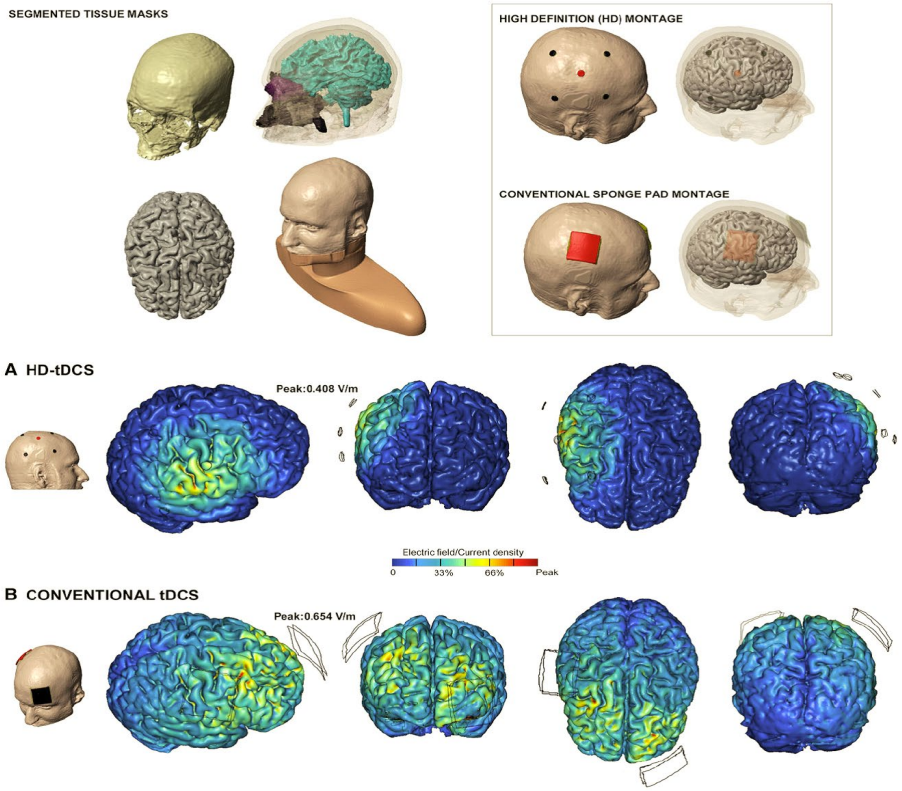


Figure 2: Electric field generated using HD-tDCS. Computational models predict brain targeting by high-definition tDCS using the 4 X 1 montage compared with conventional tDCS using a bipolar sponge montage. (Top left) Sample segmentation masks of the high resolution individualized head model. (Boxed Right Panel) The high-definition 4 X 1 montage consisted of 1 anode, positioned over the motor region, surrounded by 4 cathodes at 7 cm radius—all the electrodes were high-definition mini electrodes. The conventional sponge montage used 1 anode centered over the motor region and 1 cathode over the contralateral supraorbital region—both electrodes were conventional sponge based. (A) High-definition tDCS resulted in brain current flow restricted to within the ring with peak brain activation under the center electrodes. (B) Conventional tDCS resulted in comparatively diffuse current flow with clustering of peaks between the electrodes (not under the electrodes), (From Borckardt et al., 2011).

significantly improves upon prior un-optimized approaches using small electrodes. The analysis also reveals the optimal use of conventional bipolar montages: maximally intense tangential fields are attained with the two electrodes placed at a considerable distance from the target along the direction of the desired field; when radial fields are desired, the maximum-intensity configuration consists of an electrode placed directly over the target with a distant return electrode. If a target location and stimulation orientation can be defined by the clinician, then the proposed technique is superior in terms of both focality and intensity as compared to previous solutions and is thus expected to translate into improved patient safety and increased clinical efficacy (DaSilva et al., 2015; Dmochowski et al., 2011; Donnell et al., 2015; Edwards et al., 2013; Garnett et al., 2015; Kuo et al., 2013; Villamar et al., 2013a). HD-tDCS employs more than two small electrodes. Today, the most frequently used HD-tDCS montage is the 4X1 ring set-up, which employs a central electrode surrounded by four return electrodes arranged in a circle around the central electrode (Dmochowski et al., 2011; Edwards et al., 2013; Villamar et al., 2013b).

HD-tDCS enhances motor cortex excitability (Caparelli-Daquer et al., 2012) similar to conventional tDCS, significantly improves verbal learning and working memory in healthy individuals (Nikolin et al., 2015), improves language in patients with stroke (Richardson et al., 2015), and is well tolerated (Borckhardt et al., 2012; Brunye et al., 2014; Kuo et al., 2013; Garnett et al., 2015; Richardson et al., 2015). As with tDCS, HD-tDCS studies have also been designed to stimulate one brain area for a small number of sessions (1-10 sessions).

tDCS in MCI and Dementia

Khedr and colleagues conducted a double-blind randomized clinical trial on the efficacy of cortical direct current stimulation for the treatment of Alzheimer's disease. Their cohort included 34 AD patients, who received 10 daily stimulations with 2 mA over the left dorsolateral prefrontal cortex. The study showed that cathodal and anodal tDCS improved MMSE in contrast to sham tDCS. tDCS also reduced the P300 latency of event-related potentials (Khedr et al., 2014).

In a double-blind, cross-over, sham-controlled study, anodal tDCS was administered to the left inferior frontal cortex during task-related and resting-state functional MRI to assess its impact on cognition and brain functions in MCI. Anodal-tDCS significantly improved performance to the level of controls, reduced task-related prefrontal hyperactivity and resulted in normalization of abnormal network configuration during resting state fMRI.

Summers and colleagues conducted a meta-analysis to evaluate the effects of tDCS on cognitive and motor performance in healthy older adults. Of the 81 studies identified, 25 qualified for inclusion. A random effects model meta-analysis revealed a significant overall standardized mean difference. Five analyses on moderator variables indicated significant tDCS beneficial effects: (a) on both cognitive and motor task performances, (b) across a wide-range of cognitive tasks, (c) on specific brain areas, (d) stimulation offline (before) or online (during) the cognitive and motor tasks (Summers et al., 2016).

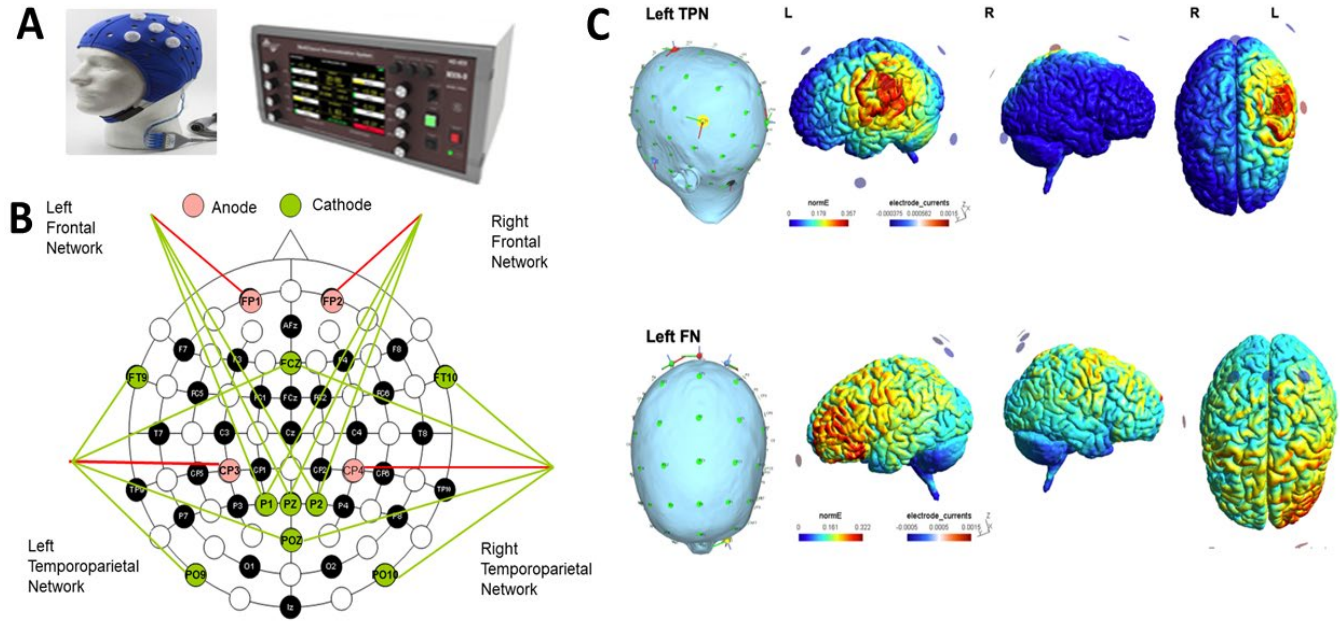


Fig 3: A. HD-TDCS devices and HD-cap. **B.** Diagram of HD-tDCS stimulation protocol with 4 networks, as applied in the study by Turski et al., 2017. Each network will be stimulated for 20 min at 1.5 mA. **C**. Visualization of current flow modeling using standard MRI and head model. It shows the left temporoparietal (TPN) and frontal (FN) networks. Heat map color corresponds to higher field intensity. Modeling done using SimNIBS software. Published in Turski et al, 2017.

A recent meta-analysis examining the effects on memory of tDCS in persons with MCI and dementia revealed a statistically significant medium effect size for immediate effects (Cruz Gonzalez et al., 2018). **Given the neuromodulatory and disease modifying potential of HD-tDCS and traditional tDCS, the question is posed whether stimulation of multiple cortical areas over months or years might elicit favorable and lasting effects on cognitive function. This might have therapeutic value for subjects with evolving dementia syndromes. Whether chronic tDCS or HD-tDCS have the potential to modify the clinical course of MCI and early dementias is unknown.**

Extended Multiple-field HD-tDCS is well tolerated and safe in healthy adults

In preparation for this trial, we studied safety, feasibility and tolerability of daily HD-tDCS over 4 brain regions for 20 sessions in healthy adults (Turski et al., 2017). Five healthy adults underwent physical and neurological examination, electrocardiogram (EKG), electroencephalogram (EEG) and cognitive screening (ImpACT) before, during and after HD-tDCS. Four networks (left/right temporoparietal and frontal) were stimulated in sequence (20 min each) using HD-tDCS in 20 daily sessions. Sessions 1-10 included sequential dose-escalating stimulation of both temporoparietal networks, sessions 11-15 stimulations of 4 networks (1.5 mA/network), and sessions 16-20 two daily stimulation cycles of 4 networks/cycle (1.5 mA/network) (Table 1). Side effects, ImpACT scores and EEG power spectra were compared before and after HD-tDCS.

	Intensity	Networks stimulated	Daily cycles	# of stimulation sessions	Duration of stimulation	Interval between cycles
Week 1	1mA	2 (R+L temporooccipital)	1	5	40 min	N/A
Week 2	1.5mA	2 (R+L temporooccipital)	1	5	40 min	N/A
Week 3	1.5mA	4	1	5	80 min	N/A
Week 4-6	1.5mA	4	2	5	160 min	2 hrs

Table 1: Outline of stimulation schedule in feasibility trial conducted by Turski et al., 2017.

All subjects completed the trial. Adverse events were tingling, transient redness at the stimulation site, feeling of being stimulated for 3 hrs and one self-resolving headache. EEG power spectrum showed decreased delta power in frontal areas several days after HD-tDCS. While at the group level ImpACT scores did not differ before and after stimulations, we found a trend for correlation between decreased EEG delta power and individual improvements in ImpACT scores after HD-tDCS.
We concluded that repeat daily stimulation of multiple brain regions using HD-tDCS is feasible and safe in healthy adults. Preliminary EEG results suggest that HD-tDCS may induce long lasting changes in excitability in the human brain.

In the proposed pilot trial, we will perform 3 monthly blocks of daily stimulations according to schedule applied in week 3 (table 1).

6.0 STUDY OBJECTIVES AND ENDPOINTS

Objectives	Endpoints
Primary	
• To assess the feasibility of MFE-HD-tDCS plus simultaneous computerized CT as a viable intervention to improve cognitive function in patients with MCI.	• Treatment completion rates (Primary) • Consent rates.
Secondary	
• To collect preliminary data on the efficacy of MFE-HD-tDCS with simultaneous computerized CT, administered in 15 sessions over a period of 3 months, to improve cognition in subjects with MCI.	• ADCS PACC score at 3 and 6 months.
• To collect preliminary data on the efficacy of MFE-HD-tDCS in combination with computerized CT, administered in 15 sessions over a	

period of 3 months to improve quality of life in subjects with MCI.	
• To collect preliminary data on the effects of MFE-HD-tDCS combined with computerized CT on brain function and on neurodegeneration.	• Neurite density (intracellular volume fraction) at 3 months • Diffusivity indices: Fractional anisotropy(FA), Mean(MD), Axial(AD), Radial(RD) Diffusivity at 3 months • Morphometric indices: Cortical thickness, Surface Area, Volume, gyrification index at 3 months • ASL perfusion indices: blood flow, blood volume, mean transit time, time to peak, at 3 months

7.0 STUDY DESIGN

7.1 General Design

The study design is a double-blinded, randomized pilot clinical trial of repetitive daily HD-tDCS/sham HD-tDCS, administered in combination with CT to subjects with MCI in 3 monthly blocks of 5 daily sessions for a total of 15 sessions. The design is outlined in figure 4.

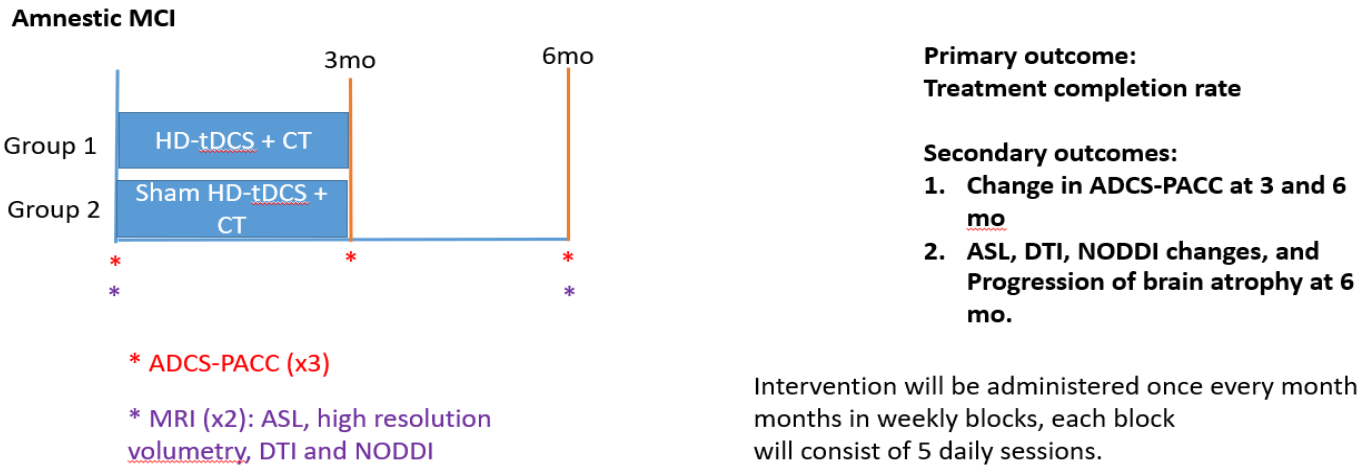


Figure 4: Design of the HD-tDCS + CT trial in subjects with MCI

Brief Description of Study Groups

20 participants with MCI, ages 50-90 years, will be assigned to one of two groups:

1. Active HD-tDCS + cognitive training (n=10)
2. Sham-HD-tDCS + cognitive training (n=10)

Recruitment outline
This is a multisite trial. Participating sites are the Medical College of Wisconsin, Milwaukee, and the University of Wisconsin, Madison.

	UW-Madison Site (n=10)		MCW Site (n=10)	
	Baseline	**Treatment and Follow-Up**	**Baseline**	**Treatment and Follow-Up**
aMCI	10	10	10	10
Total	10	10	10	10

Table 2: Recruitment outline

Information about subjects will be obtained from medical records, surveys, interview questions, data collection forms, and imaging studies.

Expected duration of intervention is 3 months for each subject and **expected duration of participation** in the trial is 6 months.

7.2 End of Study Definition

End of study will occur when 20 subjects have completed this pilot project.

8.0 SUBJECT SELECTION

8.1 Inclusion & Exclusion Criteria

Eligibility will be determined by inclusion and exclusion criteria below and confirmed by medical record review as necessary.

Inclusion Criteria
1. Age ≥50-90 years
2. Willing and able to undergo all procedures
3. Retains decisional capacity at initial visit
4. Meets criteria for MCI, amnestic type (Petersen, 2004).

Exclusion Criteria
1. Significant kidney injury requiring hemodialysis
2. Automatic Internal Cardiac Defibrillator (AICD) or Pacemaker
3. Significant congestive heart failure
4. History of clinically significant ischemic or hemorrhagic stroke, or lacune or infarct considered by radiologist likely to cause or contribute significantly to cognitive symptoms
5. History of thalamic lacunar stroke
6. Modified Hachinski Ischemia Score >4 points
7. History of seizure disorder requiring medication
8. History of brain surgery (for seizure disorder, aneurysms, or benign/malignant tumor)

9. History of HIV/AIDS
10. Severe untreated obstructive sleep apnea
11. Greater than three servings alcohol daily or illicit drug use
12. Major neurologic disorders other than dementia (e.g., MS, ALS)
13. Schizophrenia, bipolar disorder, other serious mental illnesses
14. Other significant medical conditions at investigators' discretion
15. Pregnancy
16. Lack of study partner (Participants are allowed to find a new study partner if the original study partner withdraws)

8.2 Vulnerable Populations

The consent process is conducted as a face-to-face interview with the patient. No study procedures are initiated until the consent process is complete. In all cases, prospective participants with dementia will first be assessed for their ability to provide informed consent.

The subject must provide consent himself/herself at screening. We will not enroll any participant deemed to have impaired decisional capacity. Participants will be consented at the study sites involved using local procedures established by the individual sites.

If any participant appears to have cognitive impairment when they return for their follow up visit, capacity to give consent will be assessed in clinical interviews of participants by clinicians experienced in clinical dementia research. If it is found the subject no longer has capacity to consent, the Legally Authorized Representative will provide consent if appropriate.

8.3 Subject Identification

Self-Identification

Potential subjects may self-identify by responding to IRB-approved recruitment efforts, such as web postings, posters/flyers, radio or TV ads, newspaper ads, mass mailings, email blasts, etc. IRB-approved screening scripts, eligibility questionnaires, and email response templates will be utilized when communicating with potential subjects who respond to these recruitment methods. Information collected from the potential subject is to be limited to protect the potential subject's privacy and information collected from potential subjects who fail pre-screening will be destroyed.

Identification in Clinical Practice

Potential subjects may be identified during routine visits to the Memory Disorder's Clinic in the Neurology Clinic at the Froedtert and the Medical College of Wisconsin (MCW), Middleton VA Hospital Madison, Memory Assessment Clinic [University of Wisconsin (UW) Hospital and Clinics' Memory Clinics and the Wisconsin Alzheimer's Institute (WAI) Diagnostic Clinic Network, a group of diagnostic clinics associated with the WAI experienced in diagnosing and recruiting patients with Mild Cognitive Impairment and Alzheimer's disease. A member of the clinical team will inform potential subjects of the research opportunity and provide an IRB-approved consent form. Potential subjects will be pre-screened through medical record review and conversation with the subject using an IRB-approved script. Information collected from potential subjects who fail pre-screening will be destroyed. Potential subjects who meet all pre-screening criteria will be invited for a formal screening/baseline visit.

Department Database

The Wisconsin ADRC Recruitment Registry, described under a separate UW-Madison protocol (2011-0772) may be used to identify eligible subjects.

8.4 Subject Recruitment

A total of 20 subjects will be recruited from two sites in the United States. Several recruitment strategies may be employed, and sites may use a combination of methods depending on their capabilities. Possible recruitment strategies are as follows (non-comprehensive list):

Recruitment through Clinical Practice

If the potential subject is agreeable, they will be provided contact information for the study team or the research team will initiate contact.

Posters/Flyers

Flyers announcing that volunteers are needed for a study may be posted in memory clinics of the Wisconsin Alzheimer Institute, UW Health facilities (including UW Hospital and Clinics, The American Center, South Park clinics), Meriter Hospital, Middleton Veteran's Hospital. Several key details of the study will be included in the flyer (key eligibility criteria, number and length of visits, location of study site, type of remuneration) along with a call back number for people to call in case they are interested.

Telephone Recruitment

When potential subjects contact the study team, a brief description of the study's purpose and participation requirements will be reviewed. This must also include a statement that participation is voluntary. The screener will ask the caller if s/he has any questions and whether they are interested in participating. After all questions have been answered, the study team member will ask if the potential subject is interested in proceeding to the next step in recruitment for the study (e.g., scheduling a visit to learn more and go through the consent process, or answering some screening questions).

Letters

UW Madison will utilize the Wisconsin Alzheimer's Disease Research Center (ADRC) recruitment resources to identify individuals who meet inclusion criteria. The ADRC supports researchers in their pursuit to identify answers that will lead to improved diagnosis and care for patients, as well as those seeking a cure and prevention of Alzheimer's Disease. The ADRC can hold the names and eligibility information of potential volunteers, allowing ADRC staff to contact them for appropriate research studies.

The ADRC will send the recruitment letters to eligible individuals which contain the reason for receiving the letter and outlining the procedures and purpose of the study, signed by Drs Hrissanthi Ikonomidou and Sanjay Asthana. If individuals are interested, they can contact Dr. Ikonomidou by email or phone.

8.5 Remuneration and Retention Strategies

For subjects making trips of 25 miles or more one way, they will be reimbursed at the standard UW or MCW travel rate. Remuneration will be offered for specific procedures.

MRI: $50 x 2 scans ($100)
Neuropsychological Testing: $50 x 3 evaluations ($150)
Stimulation: $20 x 15 sessions ($300)

Study partners will receive $25 for baseline and $25 for both post-treatment follow-up participation. The study partner will receive a total of $75 if they complete all visits, even if they participate via telephone.

Payments for each completed activity will be processed within 12 weeks from completion.

For MCW subjects only, on a case-by-case basis, subjects may be reimbursed for an overnight stay (up to 5 days per week) in a hotel or other form of lodging if necessary to complete a study visit. If their visit requires an overnight stay, they will receive a $25 meal reimbursement per night.

To pay subjects, we need their social security number. Any payment may be reportable as income on their taxes.

Subject's enrolled at MCW may be eligible to stay at Kathy's House as an alternative to a hotel or other form of lodging.

Kathy's House

If subjects reside at a permanent address 50 miles or greater from Milwaukee, they may be eligible for lodging at Kathy's House. Kathy's House is located on the Froedtert Hospital campus and provides housing for research subjects who require a stay greater than 3 days.

Each room includes the following:
- Most rooms have both a queen and a single bed
- Private bathroom with walk-in shower
- Bed and bath linens
- Television, small refrigerator, and telephone (local)

While lodging at Kathy's House, guests also have access to the following communal amenities:
- Fully equipped kitchen
- Refrigerator, pantry, and freezer storage space
- Free Wi-Fi
- Living Room
- Dining Room
- 6 interior & 4 exterior lounge spaces
- Fitness Center
- Meditation Room
- Laundry facilities
- Library, including computer workstations

Food
- Guests are responsible for their own meals
- Each room has designated pantry, refrigerator, and freezer storage space in the kitchen
- A meal from a local restaurant is provided once a week.

Parking and Transportation
- Parking is available for guests on a surface lot.
- Complimentary shuttle service is available during the week on a limited basis due to the COVID-19 pandemic.

Guest Responsibilities

It is important that guests staying at Kathy's House are comfortable in a communal environment. There is no maid service, so guests are asked to clean up behind themselves in the common areas and in their rooms. Rooms are thoroughly cleaned upon guest check-out.

Guests staying at Kathy's House must have a caregiver with them for the duration of their stay. Guests must also be able to perform basic mobility and care functions, including:

- do personal laundry as needed
- plan, prepare and clean up following meals

Payment for lodging at Kathy's House will be arranged and covered by the study team. Care givers are expected to lodge with subjects to the extent possible.

With the subject's verbal consent, a referral can be sent by the study team to Kathy's House. The following personal information will be included in the referral to Kathy's House: subject and care giver name, date of birth, gender, city, state, zip code, phone number, email address, and reason for visit.

Upon reception of the referral, Kathy's House will conduct a formal background check using TruthFinder.com. The subject and caregiver's names and birthdates will be used to check for any criminal charges associated with either person. Kathy's House has the right to reject any referral based on this background check.

Kathys-house.org provides additional information.

8.6 Early Termination and Withdrawal

Subjects are free to withdraw from participation in the study at any time upon request.

The Principal Investigator (PI) may discontinue or withdraw a subject from the study for the following reasons at his/her discretion:

- Pregnancy
- Subject non-compliance with study requirements (e.g., study intervention non-compliance)
- If any clinical adverse event (AE) or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the subject
- If the subject is no longer an appropriate candidate for participation
- There is evidence of progressive disease or unacceptable toxicity
- Subject unable to receive scheduled intervention for 3 weeks

Subjects who sign the informed consent form and are randomized but do not receive the study intervention will be replaced. Subjects who sign the informed consent form, are randomized and receive the study intervention, then subsequently withdraw, or are withdrawn or discontinued from the study, will not be replaced.

The following actions should be taken if a subject withdraws, or fails to return for a required study visit:

- The site will attempt to contact the subject and reschedule the missed visit within 6 weeks and counsel the subject on the importance of maintaining the assigned visit schedule and ascertain if the subject wishes to and/or should continue in the study.
- Before a subject is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the subject where possible, 3 telephone calls and, if necessary, a certified

letter to the subject's last known mailing address or local equivalent methods. These contact attempts shall be documented in the subject's medical record or study file.

- If the subject continues to be unreachable, they will be considered to have withdrawn from the study with a primary reason of lost to follow-up. The withdrawn date is the last day of attempted contact.

The study team will attempt to obtain the following information from subjects following early termination or withdrawal: Adverse events and reason for withdrawal.

9.0 STUDY AGENT (STUDY DRUG, DEVICE, BIOLOGIC, VACCINE, ETC.) AND/OR PROCEDURAL INTERVENTION

9.1 Study Procedural Intervention(s) Description

- For devices: The labeling will contain the statement "CAUTION - Investigational Device. Limited by Federal (or United States) Law to Investigational Use."

HD-tDCS Stimulation protocol

There will be two groups/arms in this trial,

a. A group that will receive HD-tDCS treatments and cognitive training (CT) (n=10),
b. A group that will receive sham HD-tDCS treatments and cognitive training (n=10).

HD-tDCS/sham HD-tDCS sessions combined with CT will be administered in monthly blocks of 5 consecutive daily sessions for a total of 15 treatments or 3 monthly blocks. For that, treatments will be administered at the MCW facilities or the UW Hospital. Study personnel may also visit subjects at their homes to administer the intervention if the subjects prefer. The safety and feasibility of home delivery of this intervention is supported by the existing literature (Im et al., 2019). Both HD-tDCS and sham-HD-tDCS treatments will be administered during a cognitive training session. The HD-tDCS procedure is described in detail below. We will sequentially stimulate or sham stimulate four networks, left and right frontal and left and right temporoparietal with 1.5mA. Each network will be stimulated/sham-stimulated for 20 min for a total of an 80 min stimulation session. On Mondays prior to CT and Fridays after CT, subjects will be assessed on competency and ability to perform the CT. The CT blocks will start after 5 min of stimulation of each network and will last 15 min/network (total 60min/session).

Figure 5: HD-tDCS devices and HD-Cap (bottom right)

Detailed description of the HD-tDCS procedure
When conducting HD-tDCS, specially designed insets, electrodes, stimulation protocols, and conductive gels will be used. Appropriate instrumentation, electrode design, and protocols are considered important for HD-tDCS safety and comfort. A flexible cap will be placed on each participant's head.

The electrode casings or holders will be secured in the cap. The skin prepping guidelines as listed in Villamar et al. (2013a) will be followed: separating the hair inside the electrode casings until the scalp is exposed, removing hair products and dirt on the scalp using alcohol swabs and then filling the electrode casings with 3 mL of Signa Gel (Parker Laboratories, NJ) or HD-GEL™ (Soterix Medical) with more applied if needed. The electrodes will be placed on a platform inside the casings so that they are completely immersed in the gel. More gel will be applied to cover the electrodes if needed, and then they will be held in place with the casing caps. Impedance values will be examined for all the electrodes and will all be verified to be <2 quality units.

The electrodes will be connected to a Soterix Medical 1x1 low-intensity DC stimulator (UW-Madison, Figure 5, top panel) or a Soterix MxN-9 High-Definition stimulator (MCW, Figure 5, bottom panel). Software packages such as SimNIBS 2.1 (Saturnino et al., 2018 bioRxiv) and those available through Soterix Medical (HD-Targets/HD-Explore) may be employed for current flow and electric field modeling for HD-tDCS. At MCW, we may use patient's structural MRI, (baseline or the one obtained for routine clinical standard of care), for modeling and/or for navigating to the HD-tDCS electrode positions from the brain surface on to the patient's scalp, using Transcranial Magnetic Stimulation (TMS).

Participants will be randomized to receive real or sham HD-tDCS. For real HD-tDCS, the device will be ramped up to 1.5 mA and maintained at this current for 20 minutes. For sham HD-tDCS, the device will be ramped up to 1.5 mA, but after 30 seconds, will be ramped back down to 0 mA and stay off for the remainder of the 20 minutes for a total of an 80 minutes of stimulation session. The MxN-9 device allows for pre-programming by an unblinded member of the study team that will not be present during the

stimulation procedures. The device operator and the study subject will therefore remain blinded to the stimulation condition.

In the case of the Soterix Medical 1X1 low-intensity stimulator, an unblinded study member will set-up the device and leave the room after covering or hiding the sham button or switch (e.g. using black tape). This will guarantee the blinding of both, the study participant and the device operator.

Each cognitive training session will last a total of 60 minutes; This will be achieved by starting cognitive training 5 minutes after the initiation of tDCS (i.e. a total of 15 minutes of cognitive training per network).

Method of electrode positioning for HD-tDCS

There are two approaches that we will explore for HD-tDCS electrode positioning: 1) electrode positioning will be the same for all patients and will be based on the International EEG 10-20 system and 2) electrode positioning may be different across patients, but guaranteeing the same cortical targets across all patients, as determined using the software packages mentioned in the earlier section (Figure 3). For #1, according to the diagram presented in figure 6 of the protocol, the positions FP1, FP2, CP3, CP4 will be the anodes for each of the four networks and 10 positions P1, PZ, P2, FT9, FT10, FCZ, POZ, PO9, PO10 will be used as cathodes. The assignment of the electrodes to the 4 different networks is outlined in the diagram in figure 3.

The placement of electrodes is based on landmarks on the skull, namely the nasion (Nz), the inion (Iz), and the left and right pre-auricular points (LPA or T9 and RPA or T10). The first step is to form the line from Nz to Iz, over the vertex (Figure 6).

To determine the location of the vertex, the contour from LPA (T9) to RPA (T10) is also passed over the vertex. These two contours should intersect at 50% of their lengths and the point thus obtained is the exact vertex. Along the sagittal Nz-Iz scalp contour over the vertex, the positions Fpz, AFz, Fz, **FCz**, Cz, CPz, Pz, POz and Oz are marked at 10% distances along this antero-posterior contour. With position Cz at 50% along this contour, corresponding to the vertex, the position of Oz is at a distance of 90% from Nz and 10% from Iz.

Along the coronal LPA-RPA scalp contour over the vertex, the positions at 10% above the LPA and the RPA are marked. These positions are necessary to determine the horizontal contours over the left and right temporal lobe.

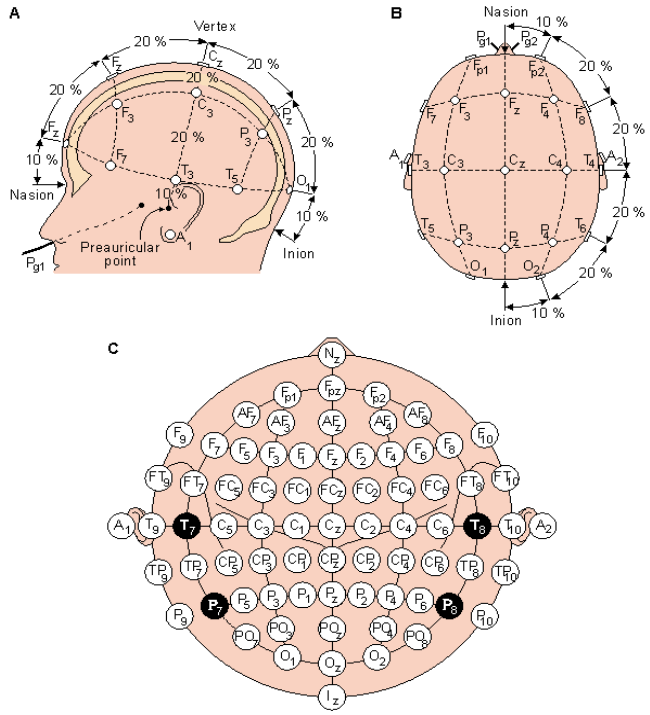
A horizontal circumferential contour is determined over the left temporal lobe from Fpz to Oz, through the location which was marked at 10% above LPA. Along this contour, the positions **Fp1**, AF7, F7, FT7, T7, TP7, P7, PO7 and O1 are determined at 10% distances. The circumferential contour over the right temporal lobe is determined in the same fashion from Fpz to Oz over the location 10% above RPA, and the positions **Fp2**, AF8, F8, FT8, T8, TP8, P8, PO8 and O2 are marked at 10% distances.

Figure 6: Method of electrode placement according to the 10/10 system.

A horizontal circumferential contour is determined over the left side of the head between T9 (LPA) and Nasion (Nz). Along this contour, the position **FT9** is determined at 20% distances. The circumferential contour over the right side is determined in the same fashion, this leads to placement of electrode **FT10.** A horizontal circumferential contour is determined over the left side of the head between T9 (LPA) and Inion (IZ). Along this contour, the position **PO9** is determined at 40% distance from the IZ. The circumferential contour over the right side is determined in the same fashion, this leads to placement of electrode **PO10**.

The positions for anodal electrodes **Fp1, Fp2, CP3** and **CP4** and the cathodal electrodes **P1, Pz, P2, PO9, PO10, FT10, FT9, FCZ, POZ** are marked with a waterproof permanent marker using **red color for the anodes and black color for the cathodes.**

The markings will be maintained and refreshed from session to session to minimize need for new measurements. Whenever the markings fade, new measurements using the same landmarks will be performed in a stereotypic fashion.



The devices used are made by Soterix Medical and were used in the previous adult studies without incident. They are marked as Investigational Use Only. The first set of devices are 1) a low-current low-voltage tDCS sources; 2) a tDCS splitter box. The low-voltage low-current source generates a low intensity current for the duration of the stimulation. It is powered by 9V batteries and limited to 2 mA maximum. In this study we will use intensities of 1.5 mA or below as a safety factor. The splitter does not actually generate any stimulation. It can be thought of as a passive splitter box. For #2, we will use the MxN-9 Soterix stimulator; note that a splitter box is not needed with this device. Modelling will be performed on every patient taking into account the size and shape of the head and the skull thickness. If the anticipated peak electric field at 1.5 mA exceeds 0.654 V/m in the brain tissue, the current will be reduced from 1.5 mA to a lower calculated value, so that the maximal field intensity of 0.654 V/m is not exceeded. Peak electric field of 0.654 V/m is observed in adults who receive tDCS with 2mA total current (Figure 2; Borckardt et al., 2011).

The output cable of the splitter box or the MxN-9 device connects to the STENs electrodes, which are immersed in the SIGNA gel or HD-GEL that is held in place by the plastic insets (or electrode casing caps), which are mounted on to the cap.

Dermal abrasion is not typically required for a good contact, however, gentle moving of hair and working of gel into skin might be used – Just as done for EEG.

Cognitive training

We will use the Posit Science's brain plasticity-based BrainHQ exercises platform (*https://www.brainhq.com*). The BrainHQ platform consists of more than two dozen exercises grouped into six broad categories: Attention, Memory, Brain Speed, Intelligence, People Skills, and Navigation. These general categories clearly overlap and the assignment of any given game to one single *spec*ific category is a matter of predilection for a cognitive domain, and not a matter

of absolute specificity (e.g., a game under the 'Memory' category will inevitably recruit 'Attention' and 'Brain speed' mechanisms, both cognitive functions necessary for adequate memory formation).

All participants will receive active Computerized CT which will be administered for 60 min during HD-tDCS or sham HD-tDCS sessions.

Participants will engage in exercises of increasing complexity designed to train attention, memory, processing speed, people skills and navigation. To maximize the synergistic effect of HD-tDCS and CT on the same brain region, we plan to couple the electric stimulation of a network with a game that has a preferential effect on that same network (e.g. left temporoparietal stimulation with a verbal memory task, and right temporoparietal stimulation with a non-verbal memory task).
For this purpose, we will select 12 games (out of an approximate total of 29) and assign each one of them to one of the four possible networks that will be stimulated (see table 3).

Table 3: Training Schedule

Brain Network	Monday	Tuesday	Wednesday	Thursday	Friday
Left frontal	**1)** Divided attention **2)** Mixed signals **3)** Mind bender	**1)** Divided attention **2)** Mixed signals **3)** Mind bender	**1)** Divided attention **2)** Mixed signals **3)** Mind bender	**1)** Divided attention **2)** Mixed signals **3)** Mind bender	**1)** Divided attention **2)** Mixed signals **3)** Mind bender
Left temporo-parietal	**1)** memory grid **2)** Face-facts **3)** In the know	**1)** memory grid **2)** Face-facts **3)** In the know	**1)** memory grid **2)** Face-facts **3)** In the know	**1)** memory grid **2)** Face-facts **3)** In the know	**1)** memory grid **2)** Face-facts **3)** In the know
Right frontal	**1)** Eye for detail **2)** Target Tracker **3)** Double decision	**1)** Eye for detail **2)** Target Tracker **3)** Double decision	**1)** Eye for detail **2)** Target Tracker **3)** Double decision	**1)** Eye for detail **2)** Target Tracker **3)** Double decision	**1)** Eye for detail **2)** Target Tracker **3)** Double decision
Right temporo-parietal	**1)** Hear, Hear **2)** Right turn **3)** Recognition	**1)** Hear, Hear **2)** Right turn **3)** Recognition	**1)** Hear, Hear **2)** Right turn **3)** Recognition	**1)** Hear, Hear **2)** Right turn **3)** Recognition	**1)** Hear, Hear **2)** Right turn **3)** Recognition

Choosing 12 games out of a larger pool of 29 will allow us to narrowly select those games better suited for the stimulation of the 4 proposed brain networks for every block (3 games/network). The selection of 12 games will also facilitate scheduling (3 games/network/day).

The combined HD-tDCS/sham HD-tDCS and CT sessions will be performed monthly on 5 consecutive days for 3 months. A study coordinator will visit each participant at their homes or meet them in the hospital for a session. The laptop with the appropriate software will be provided by the investigator team.

The weekly training schedule is outlined in table 3. A 'Personalized Trainer' option will present an automated sequence of the chosen exercises. Participants will have identical sessions initially, but

later they will be given different sessions based on their individual performance history (3 games/network will always remain a constraint). The active training blocks will each last 15 min.

Procedures for Training of Clinicians on Procedural Intervention

The study interventions will be administered by a research specialist or the study coordinators who will be trained by Drs Granadillo, Shah-Basak, Ikonomidou, and Hancock in administering HD-tDCS and cognitive training.

9.2 Method for Assigning to Treatment Groups

Randomization to the two arms will be the responsibility of an unblinded member of the study team.

9.3 Unblinding Procedures

Unblinding will be done in emergent circumstances where the identity of the treatment needs to be known. All efforts will be made to maintain blinding except in the case of urgent medical necessity.

9.4 Study Intervention Compliance

Threshold adherence/compliance is achieved when the enrolled participant completes all HD-tDCS/sham HD-tDCS + CT sessions and undergoes cognitive testing at 0, 3 and 6 mo. This degree of participation will allow calculation of the primary outcome, i.e. treatment completion rates.

9.5 Concomitant Therapy

Permitted Concomitant Therapy

Subjects will continue all medications and other prescribed treatments during participation in this study. No life style changes are necessary.

Prohibited Concomitant Therapy

None

10.0 STUDY VISITS AND PROCEDURES

10.1 Study Calendar

The procedures performed at each study visit are listed in the table below.

	Baseline	Treatment Period			Month 3	Month 6	Early Withdrawal
Visit	**1**	**2-6**	**7-11**	**12-16**	**17**	**18**	
Visit Window	**-6 months**		**±4 weeks**	**±4 weeks**	**Up to 6 weeks post Visit 16**	**3 months post Visit 16 ± 8 weeks**	
Timeframe		**Week 1 (Month 1)**	**Week 2 (Month 2)**	**Week 3 (Month 3)**			
Informed Consent	X						
Review Eligibility Criteria	X						
Demographics	X						
Review Concomitant Medications	X				X		X
Obtain Medical History	X						
Physical Exam	X				X		X
Vital Signs[1]	X				X		X
Randomization	X						
Study Partner Questionnaires	X				X	X	
Cognitive Battery	X				X	X	
MRI	X				X[2]		
TMS Neuronavigation (for stimulation planning) MCW only	X						
Device Administration		X	X	X			
Adverse Events Review/Assessment		X	X	X	X	X	X

1 Vitals include: pulse rate, body temperature, blood pressure, respiration rate, height, and weight
2 MRI will aim to be completed within 6 week window, but can be completed up to 12 months after Visit 16 if scheduling problems arise

10.1 Screening and Enrollment

Subjects may complete study procedures at both sites (e.g., baseline MRI at UW and Month 3 MRI at MCW) and any individual subject's data may be collected between sites depending on subjects' ability to travel between sites, study team availability, and investigator approval. Subjects may be dispersed interchangeably and unevenly between sites based on, for instance, subject convenience, study team availability, the site's ability to collect data, and/or the study team's preference to complete datasets. If a subject is unable to complete all study procedures prior to the end of study funding, they may choose to complete remaining study procedures at the main site if deemed eligible by the study investigator

The Screening and Enrollment visits and procedures are described in detail below.

Pre-screening

Participants will be screened by telephone for major inclusion and exclusion criteria. Eligibility may be determined by screening existing records. Some ineligibility criteria may become known during the course of the study, for example, development of new medical conditions or abnormality detected on brain imaging that may change the participant's eligibility.

Decisional Capacity

It should be noted that all participants enrolled must retain decisional capacity at their baseline visit. The only situation where we indicate a plan to obtain assent from a participant is when a patient with MCI loses capacity over the course of the study and has indicated to their legally authorized representative that they wish to continue in the study even after losing decisional capacity. In this case, assent will always operate as a veto to the subject's participation, despite the surrogate's preference. Assent will always be verified prior to and during any study procedures.

Baseline Visit

Prior to any procedures, informed consent will be obtained. Visit procedures may take up to 7 hours but may be more or less depending on time needed for breaks, participant comfort, and scanner set-up. This baseline visit will likely be completed over 2-3 days but can be completed over multiple days prior to Visit 2.

Once consent is obtained, the following assessments will be completed, and information collected for study participants.

- Document informed consent
- Review eligibility based on inclusion/exclusion criteria
- Demographics (age, sex, race, ethnicity, age, year of birth)
- Medical history
- Physical and Neuro exam including vitals
- Medications
- Cognitive battery
- Structural MRI
- TMS Neuro-navigation (MCW only)
- Study Partner Questionnaires

Vitals and Medical Evaluation

Vitals include height and weight measurements, resting blood pressure, body temperature, heart rate, and respirations. Physical and neurological examinations will be conducted by clinicians and include a review of systems.

Interviews and questionnaires:

Participants will be interviewed by staff for completion of questionnaires intended to clarify medical history and level of awareness of cognitive deficits.

Study partners will complete three questionnaires designed to assess the subject's cognitive function and ability to perform activities of daily living. These questionnaires include:

- Alzheimer's Disease Cooperative Scale - Activities of Daily Living – Mild Cognitive Impairment (ADCS-ADL-MCI)
- Lawton-Brody Instrumental Activities of Daily Living Scale (Lawton IADL)
- Short Form of the Informant Questionnaire on Cognitive Decline in the Elderly (Short IQCODE)

Cognitive Battery

Participants will complete a broad battery of neuropsychological tests administered by trained personnel; this comprehensive battery will include the Alzheimer's Disease Cooperative Study-Preclinical Alzheimer Cognitive Composite (ADCS-PACC), which will serve as the primary outcome measure for the future phase II trial (see Table 5). The ADCS-PACC is a composite of 4 measures with well-established sensitivity for the detection of cognitive decline in prodromal and mild dementia. The final score is determined from its components using an established normalization method; the four Z scores are then added to form the composite. Consequently, a change of 0.5 standard deviations on each component would correspond to a 2-point change on the composite. We will alternate between 3 different versions of the test in order to minimize the risk of learning effects (Donohue et al., 2014).

The ADCS-PACC emerged as a response to concerns about ceiling effects, and the insensitivity to early stage deficits and to cognitive change of more standard Alzheimer's disease measures (e.g. ADAS-Cog). The PACC is currently being used as the primary outcome measure in multiple ongoing studies of early or preclinical Alzheimer's disease (Donohue et al., 2014; Harrison, 2018).

Table 5. ADCS-PACC

Cognitive Subdomain	Test
Episodic Memory	Rey Auditory Verbal Learning Test (RAVLT) (or Free and Cued Selective Reminding Test (FCSRT) (0-48 points)
	Logical Memory IIa sub-test from the Wechsler Memory test (0-25 points).
Orientation	Mini Mental Status Examination (MMSE) (0-30 points).
Executive function	Digit Symbol substitution test (DSST) from the Wechsler Adult Intelligence Scale-Revised (0-93 points).

The neuropsychological test battery will include a multidimensional set of brief measures assessing cognitive, emotional, motor and sensory function that can be used as a "common currency" across diverse study designs and settings which will be supplemented by measures that are consistent with other national studies of Alzheimer's disease (see Table 6).

Table 6. Cognitive Battery (Secondary Outcome Measures)

Domain	Subdomain (measure name)	Test
Cognition	Premorbid Estimate	Wide Range Achievement Test-4 (WRAT-4)
	Attention & Information Processing Speed	Trail Making Test
	Executive Functioning	Clock Drawing Test Controlled Oral Word Association Test (letter & category)

		Wisconsin Card Sorting Test (WCST)
	Language Functioning	Boston Naming Test (BNT), Language Screen
	Motor Skill	Grooved Pegboard
	Visuospatial Skill	Judgment of Line Orientation (JLO)
	Memory	Benton Visual Memory Test-Revised (BVMT-R)
Emotion (Self-Report)	Mood	Geriatric Anxiety Inventory (GAI) Geriatric Depression Scale (GDS)
Validity	Effort	Rey 15 Item Test

Magnetic Resonance Imaging (MRI)

MRI Safety Screening: Site-specific MR safety screening procedures will be followed. All participants will be screened for medical devices, implants, and metal prior to undergoing MRI, first on the telephone, and again prior to entering the scanner room. If it is necessary to review medical records to confirm contraindication, a review of medical records (e.g. previous surgeries) will take place prior to the scheduled visit. Prior to entering the MRI scan room, an x-ray exam may be completed as a safety precaution on subjects who might have metal in body (for example, an orbital x-ray, or x-ray of another body part). During the telephone screening process, participants will also be questioned about their ability to temporarily remove transdermal patches (such as birth control or nicotine patches). Women of child-bearing potential will be asked to confirm that they are not pregnant when signing the Informed Consent Form. If a woman has concerns or is uncertain of her pregnancy status, she will be excluded, as the risks of an MRI scan to pregnant women are currently unknown. The risks are minimal for a properly administered visit. The MR technicians are trained and prepared to deal with any problems that may arise.

We will collect a full suite of non-invasive anatomical and blood flow scans using GE 3.0 high-field scanners running the newest operating system and a 32-channel head coil. All participants are prepped and trained prior to the procedure. The MRI protocol will consist of anatomical and other advanced scans including ASL perfusion and DTI. These scans will be conducted over one session which will take approximately 60 minutes including set-up and instructions but could take longer depending on participant set-up time or need to repeat instructions. The sequences, protocols, parameters, and/or scan order may be modified during the study based on new scientific information or technical factors.

The MRI scanner is an Investigational Device of Non-Significant Risk: The MRI scanner to be used in the study is considered an investigational device due to the investigational software used in the study that was developed by the collaborators at both MCW and UW-Madison. However, the Magnetic Resonance Imaging (MRI) device is an FDA cleared device for safe and non-invasive imaging of the interior of the human body. The MRI scanner is also considered an investigational device when the 32-channel head coil is used. Modified pulse sequences include T1-weighted MPnRAGE, pseudocontinuous arterial spin labeling (pcASL), and simultaneous multislice (SMS) echo planar imaging for diffusion-weighted imaging (DWI). These pulse sequences were developed by medical physics experts using the EPIC pulse sequence development environment provided by GE Healthcare, which constrains the pulse sequences to operate within the FDA limits for safe operation with respect to gradient switching (dB/dt) and RF power (SAR). The proposed modified MRI pulse sequences (developed at MCW and UW-Madison), and the 32-channel head coil are not implantable devices, are not intended to support or sustain human life; are NOT for a use of substantial importance in diagnosing, curing, mitigating, or treating disease or otherwise preventing impairment of human health and they do not present a potential for serious risk to the health, safety, or welfare of a subject. The scanner also restricts research software from exceeding FDA safety levels. The scanner monitors the specific absorption rate (SAR) for research scans just as it does for

all other scans. Thus, the scanner with the investigational software fully engaged operates, from a technical design and functional standpoint, as a non-significant risk device in accordance with 21 CFR 812. By staying below these limits, the operating conditions of the MRI device are generally deemed, in and of themselves, to make the MRI device a non-significant risk device.

The Nova Medical 32-channel head coil (model number NMSC075-32-3GE-MR750) is an investigational device not FDA approved for clinical use. This coil device includes multiple features for safe operation involving human studies. The 32-channel coil is designed and constructed as a receive-only detector of RF signals that are emitted by the brain following the RF excitation generated by the GE MRI scanner, which is FDA approved. During the RF excitation by the scanner, the coil device is decoupled (made inactive) through redundant circuitry, thus the coil device never transmits RF to the subject, so it has no impact on subject risk or safety.

More specifically, the coil design and construction include the following safety features: (1) High voltage breakdown (>2kV) UL-94V0 flame retardant housing. (2) Rugged construction to assure safe operation in case of rough handling. (3) Active detuning circuitry providing greater than 35 db isolation per element. (4) High power passive detuning circuits in case primary detuning circuitry fails. (5) Multiple common mode traps in all receive coil cables. (6) Minimum of 5mm spacing between coil conductors and patient contact.
Additionally, the coil was designed and manufactured under an ISO 13485 certified quality management system. As part of this quality system, Nova Medical has conducted a Failure Means and Effects Analysis (FEMA) of this product and we feel that it is a non-significant risk under foreseeable normal conditions when used on the 3T GE X750 MRI scanners at MCW and UW-Madison.

Mock Scanner Training. Participants will be offered the option to engage in a mock scanner training session. The purpose of this session is to acclimate participants to the scanner and to assess their comfort level, minimize any anxiety, and provide practice and training to remain still. Training in the mock scanner will take approximately 20 minutes and will occur prior to their imaging sessions. A visual feedback system will be employed to inform the subject about her or his performance of remaining stationary. Further, contoured padding will be employed to physically ensure subject stability throughout the imaging session. After the session, any questions or concerns will be discussed. Participants that have been previously engaged in MRI as part of research may be excused from the mock scanner training.

10.2 Treatment and Follow-up Visits

After subjects have been enrolled, the On-Study/Follow-up visits and the procedures performed at each visit are described in detail below.

Treatment
Visits 2-16*

At these visits, the following will occur:

- Cognitive training with HD-tDCS treatment OR
- Cognitive training with sham HD-tDCS treatment
- On Mondays prior to CT and Fridays after CT, subjects will be assessed on competency and ability to perform the CT.

These visits will likely last about 2.5 to 3 hours.

*Missed or rescheduled treatment visits: Because there may be a benefit from a single treatment session at any point during the study, subjects who miss or need to reschedule treatment visits will be eligible to complete their missed visit at any point prior to Month 3 procedures.

Visit 17 (Month 3)

At this visit, the following procedures and tests will occur:

- Neuro and physical exam
- Vitals signs
- Cognitive battery
- Study Partner Questionnaires
- Structural MRI (will aim to be completed within 6 week window, but can be completed up to 12 months after Visit 16 if scheduling problems arise)

This visit will be approximately 6 hours and will likely be completed in 1-2 days but can be completed over multiple days.

Visit 18 (Month 6)

At this visit, the following procedures and tests will occur:

- Cognitive battery
- Study Partner Questionnaires

This visit will be approximately 4 hours and will likely be completed in 1 day but can be completed over multiple days.

10.3 Unscheduled Visits

The reason for an unscheduled visit will be an unexpected or serious adverse event.

10.4 Early Termination/Withdrawal Visit

Subjects who are either withdrawn or terminated early from the study will have one final visit to report adverse events.

11.0 DATA COLLECTION, HANDLING, SHARING, AND RECORD KEEPING

11.1 Data Collection

Data Collection Forms

Standardized data collection forms (e.g., source documents, case report forms, standardized assessment forms, etc.) are used to ensure data collected are consistent and compliant with the protocol and IRB application.

Data collection is the responsibility of study team members under the supervision of the Principal Investigator (PI). The PI is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the recorded and reported data.

Data collection forms are maintained in the subject files and retained as described in Section 11.3: Records Retention.

11.2 Confidentiality and Privacy

Participants' privacy will be protected with the utmost care. We recognize the diagnosis of MCI or Alzheimer's disease, may cause embarrassment and/or discrimination. We will handle all contacts with participants with special attention to privacy. General mailings (not related to specific appointments) will be sent in discrete envelopes. Personnel making phone contacts will be trained to protect privacy when leaving messages or talking with family members who answer the phone. All research procedures will be conducted in private settings, and only information absolutely necessary to conduct our research will be gathered.

Information about study subjects will be kept confidential and managed according to HIPAA requirements. All subjects will sign a combined informed consent and HIPAA authorization form that includes specific privacy and confidentiality rights. Study data will be maintained per federal, state, and institutional data policies.

The investigator(s) will ensure that the identities of subjects are protected by using coded subject information. The log of subject identifying information that links subjects to their study-specific identification number will be maintained by the investigator. The log and all study records will be maintained in locked spaces and access will be limited to the PIs and coordinators. Electronic study records/files will be stored on a dedicated server and accessed via networked computers that are password-protected with access provided only to authorized study personnel.

11.3 Records Retention

It is the investigator's responsibility to retain study essential documents for a minimum period of 7 years following completion of the study per MCW and UW-Madison institutional policy.

11.4 Protocol Deviations

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

It is the responsibility of the Principal Investigator/site investigator/study staff to use continuous vigilance to identify and report deviations. The Principal Investigator is responsible for assessing whether the deviation constitutes noncompliance as defined by the reviewing IRB and if so, reporting it within the required time frame(s). The site investigator is responsible for knowing and adhering to the reviewing IRB requirements.

12.0 STUDY ANALYSIS

12.1 Statistical Hypotheses

- **Primary Efficacy Endpoint(s):**
  Treatment completion rates.

- **Secondary Efficacy Endpoint(s):**

- Consent rate.
- ADCS-PACC score at 3 and 6 months
- Neurite density (intracellular volume fraction) at 3 months
- Diffusivity indices: Fractional anisotropy (FA), Mean(MD), Axial(AD), Radial(RD) Diffusivity at 3 months
- Morphometric indices: Cortical thickness, Surface Area, Volume, gyrification index at 3 months
- ASL perfusion indices: blood flow, blood volume, mean transit time, time to peak, at 3 months

12.2 Sample Size Justification

Given the pilot nature of this study, two feasibility outcomes will be included: Consent rate and treatment completion rate. 20 patients are enough to calculate a 90% exact binomial confidence interval of (.75, 1) if all 20 are observed to complete treatment. Thus, this sample size is large enough to potentially exclude completion rates of 3/4 or lower. The pilot data from this study will provide initial estimates of the variability and the effect sizes; This information will then be used for the formal calculations of power and sample size necessary to conduct a future phase II trial.

This pilot study, if successful, would prove the feasibility of the proposed approach. Treatment completion rates of less than 75% could be reasonably excluded if all 20 patients are observed to complete treatment. Additionally, recruiting and consenting 20 patients over a period of 3 months would support the feasibility of conducting a larger-scale, 2-site, phase II trial with 120 MCI patients over a span of 5 years (i.e. approximately 32 patients/year). This would be the necessary next step in the path to using combined HD-tDCS/CCT as an intervention that could lead to tangible clinical improvements. This future trial would use a 2 X 2 factorial design that would allow for the inclusion of a 'true' control group (i.e. sham HD-tDCS/sham CCT). The stimulation period would be extended (six months instead of three) with the hope of inducing longer lasting effects that could translate into a slowing of disease progression; Accordingly, we would evaluate the effects of the intervention at 6, 12 and 18 months. MRI changes would be assessed at 12 months, as well as the effect on cerebrospinal fluid (CSF) AD biomarkers of a subset of patients. **Sample size justification for future phase II trial (N=120)**. Driven by the efficacy outcome of change in ADCS-PACC score from baseline to 12 months. We presume that 10% of these patients are lost to follow up before one year. We further presume that the main aim will be addressed by a two-sided test at the .05 level of the hypothesis that the improvement rate in the true HD-tDCS group exceeds that of the sham group. Under these assumptions there is a minimum 90% power to detect a 31% difference in improvement rates. Thus, this design has good power to find a moderate effect size of HD-tDCS treatment.

12.3 Subject Population(s) for Analysis

In a future phase II trial, all analyses will be done under the intent-to-treat principle as far as allowed by patient consent and follow-up. All consenting patients will be followed even if they are noncompliant. Potentially confounding patterns of loss to follow-up will be investigated via sensitivity analyses [Panel on Handling Missing Data in Clinical Trials, Committee on National Statistics Division of Behavioral and Social Sciences and Education US National Research Council. The Prevention and Treatment of Missing Data in Clinical Trials. National Academies Press, 2010].

12.4 Statistical Methods

In a future phase II trial (and for this feasibility trial as appropriate), baseline clinical and demographic characteristics will be tabulated for subjects assigned to the four treatment modes in a future 2 X 2 factorial design. Comparison of these characteristics between the four groups will be carried out with t-tests and chi-square tests. If important differences are found between the groups at baseline, the statistical analyses will be repeated after adjusting for these differences. These analyses will be considered to be supportive however.

All analyses will be done under the intent-to-treat principle as far as allowed by patient consent and follow up. All consenting patients will be followed even if noncompliant. Potentially confounding patterns of loss to follow up will be investigated via sensitivity analyses.

The primary outcome variable is efficacy, defined as improvement over time in ADCS-PACC scores from baseline to 12 mo. This will be analyzed via a standard 2 x 2 x 2 analysis of covariance,[86] where the three two-level factors are the two treatments and MCW vs. UW-Madison site, and the continuous covariate is the value of the outcome at baseline. Explicitly,

$\mu_i = \beta_0 + \beta_{HD\text{-}tDCS} \times I(HD\text{-}tDCS_i) + \beta_{train} \times I(train_i) + \beta_{inter} \times I(HD\text{-}tDCS_i) \times I(train_i) + \beta_{base} \times Y_{i,base}$,

where: μ_i is the mean outcome, indexed by *i* for subject; β_0 is the intercept term, $\beta_{HD\text{-}tDCS}$ is the HD-tDCS main effect term, I(HD-tDCS$_i$) is an indicator variable for the ith patient taking the value of 1 if s/he received true HD-tDCS and 0 for sham; β_{train} is the cognitive training main effect term; I(train$_i$) is an indicator variable for true vs. sham cognitive training in the ith patient; β_{inter} is the interaction term between the two treatments, measuring their synergy; β_{base} is the coefficient for baseline outcome level; and $Y_{i,base}$ is the baseline outcome level for patient i. For the primary aim the outcome would be cognitive function measured by ADCS-PACC score at 12 mo (since baseline ADCS-PACC score is included, this is formally equivalent to change in 12-mo ADCS-PACC score from baseline).

All hypotheses in the primary and secondary aims could be examined by testing appropriate parameters using the relevant endpoints in the above model. The primary aim of examining overall 12-mo HD-tDCS will be effected by estimating $\beta_{HD\text{-}tDCS}$ and β_{inter}, accompanied by confidence intervals and a 2-degree-of-freedom test that they are both 0. Rejection of such a test is evidence that HD-tDCS does affect patient's 12-month cognition. Similarly, therapy's effectiveness can be examined in this population by estimating β_{train}. The interaction parameter β_{inter} measures the two treatments' synergy, the degree to which CT potentiates the effects of HD-tDCS. Since all study outcomes are continuous, models will be fit using ordinary least squares.

The full 18 mo longitudinal trajectory will be modeled using a repeated measures Laird-Ware formulation (Fitzmaurice, GM, Laird, NM, and Ware, J. *Applied Longitudinal Analysis, 2nd Edition*. Wiley; New York, NY. 2011), equivalent to that stated above but with intra-subject error terms added and average changes modeled instead of single 12 mo values as fixed effects.

Analysis of cognitive data: To analyze the effect of training on outcome measures, a 2 x 4 mixed-factor analysis of variance (ANOVA) will be employed, including a within-subject factor of test performance over time (pre- vs. post-training) and a between-subjects factor of group assignment. We will use Bonferroni correction to conservatively control for familywise error. If analyses reveal that the groups were not equivalent at baseline, we will employ statistical covariates in order to control for group differences.

We will utilize adherence data to determine how many participants follow the training schedule. Threshold adherence is achieved when the enrolled participant completes all HD-tDCS/sham HD-tDCS + CT/sham-CT sessions and undergoes cognitive testing at 12 mo. This degree of participation will allow calculation of the primary outcome, i.e. change in ADCS-PACC at 12 mo. We will exclude data from participants who did not meet the minimum threshold of adherence to the interventions.

Finally, if statistically significant improvements in neuropsychological outcome measures are shown, will conduct follow-up analyses using reliable change index (RCI) scores. The RCI allows for an assessment of the magnitude of change of scores for an individual that are not susceptible to group means and standard deviations. This process has been described in detail elsewhere (Hinton-Bayre, 2010; Maassen et al., 2009). We will use the Jacobson–Truax method with a 0.90 confidence interval, which indicates a 95% chance of true improvement for anyone who passes the threshold (Jacobson & Truax, 1991).

Neuroimaging: In a future larger-scale phase II trial We will perform Kruskal- Wallis tests, to determine if there are significant between group differences at 12 mo, on each of the NODDI (neurite orientation dispersion (OD) index, neurite density (or intracellular volume fraction) (Vic),) and diffusivity (FA, MD, RD) measures, as well as measures of neurodegeneration measured by cortical thickness and ASL perfusion (cerebral blood volume, mean transit time, time to peak etc). Post-hoc analyses comparing the sham group to each of the three remaining groups will be done

using Mann-Whitney U tests. All analyses will be done with and without controlling for possible confounding factors such as age, intracranial volume, family history of AD or other dementias.

12.5 Planned Interim Analysis

There will not be an interim analysis.

13.0 RISK/BENEFIT ASSESSMENT

13.1 Potential Benefits to the Subjects

There are no direct benefits to participating in the study. The scientific benefits are great and include a better understanding of the associations between the planned interventions and brain/biomarker/cognitive changes associated with MCI and Alzheimer's disease and possible mechanisms underlying these associations. Study completion may provide evidence in support of HD-tDCS + CT as an efficacious approach to the prevention of Alzheimer's disease and related neurodegenerative disorders in midlife.

13.2 Known Potential Risks

MRI: Participants are screened by study staff prior to the scan to verify they do not have any contraindications for MRI. Potential side effects of the scan include anxiety due to claustrophobia and/or noise. Participants with known anxiety or claustrophobia will not be recommended to participate since the head must be placed fully inside the MRI scanner tube. If a participant experiences anxiety, they will be removed from the scanner and offered reassurance by study staff. To minimize the level of noise, all subjects will be fitted with disposable earplugs. Communication with the participant is possible during the scan. In addition, fatigue and physical discomfort due to the length of the scan session are possible. Participants will be reminded that the procedure is optional.

The MRI scanner to be used in the study is considered an investigational device due to the investigational software and equipment used in the study (for blood flow imaging), which is being supplied by GE; however, the baseline Magnetic Resonance Imaging (MRI) device is an FDA cleared device for safely and non-invasively imaging the interior of the human body. The scanner restricts research software from exceeding FDA safety levels. Thus, the scanner with the investigational software and equipment fully engaged operates, from a technical design and functional standpoint, as a **non-significant risk** device in accordance with 21 CFR 812.

Risks of extended neuropsychological testing: Extended neuropsychological testing may cause embarrassment, sadness when asked about personal feelings. The subject may feel bored, nervous, embarrassed with some of the tests or when answering questions about how he/she feels.

Risk of Breach of Confidentiality: Because personal information will be retained in a computer database, there is a small possibility that this information could become available to unauthorized persons. This study involves protections to minimize the chance of any such breach.

Known Interventional Risks

Risks of HD-tDCS include redness and skin irritation at the site of stimulation. When current flow is initiated the subject may perceive a tingling sensation on his/her scalp. In one study, fatigue was reported as a side effect of tDCS. Turski et al (2017) reported that one subject developed a headache during one session, which resolved when stimulation was stopped. Another subject reported poor sleep one night, which however resolved and was judged to not be caused by the stimulation.

13.3 Risk/Benefit Analysis

The risks of the proposed procedures are minimal, and the potential knowledge gains are substantial, making the risk/benefit ratio low. These experiments will provide a unique opportunity to determine the potential for HD-tDCS and CT to benefit participants with MCI.

14.0 DATA AND SAFETY MONITORING

14.1 Adverse Event (AE) Definition

Adverse event (AE) means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related.

14.2 Serious Adverse Event (SAE) Definition

An adverse event is considered "serious" if, in the view of either the investigator or sponsor, it meets any of the following criteria:

- Results in death
- Is life-threatening
- Requires an inpatient hospitalization or prolongation of an existing hospitalization
- Results in persistent or significant disability or incapacity.
- Results in a congenital anomaly/birth defect.
- A medical event, based on appropriate medical judgment, that is believed to jeopardize the subject and/or requires medical or surgical intervention to prevent one of the outcomes defining a SAE. For example: allergic bronchospasm requiring intensive treatment in an emergency room or at home, convulsions that may not result in hospitalization

14.3 Classification of an Adverse Event

Severity of Event

All AEs will be assessed by the clinician using the Common Terminology Criteria for Adverse Events (CTCAE), version 5.0, each event searchable using the Safety Profiler website (https://safetyprofiler-ctep.nci.nih.gov/CTC/CTC.aspx). For AEs not included in the protocol-defined grading system, the following guidelines will be used to describe severity.

Mild (Grade 1)	Events require minimal or no treatment and do not interfere with the subject's daily activities.
Moderate (Grade 2)	Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
Severe (Grade 3)	Events interrupt a subject's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating.
Life Threatening (Grade 4)	The subject was at risk of death at the time of the event.
Fatal (Grade 5)	The event caused death.

Relationship to Study, Study Procedure(s) and/or Study Intervention(s)

For all collected AEs, the clinician who examines and evaluates the subject will determine the AE's causality based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below.

Definitely Related	Clearly related to the study procedures/intervention and other possible contributing factors can be ruled out.
Probably Related	Likely related to the study procedures/intervention and the influence of other factors is unlikely.
Possibly Related	Possibly related to the study procedures/intervention and there are other factors that could be equally likely.
Unlikely to be related	Doubtfully related to the study procedures/intervention and there is another likely cause.
Unrelated	Clearly not related to the study procedures/intervention and/or evidence exists that the event is definitely related to another cause.

Expectedness for Study, Study Procedure(s) and/or Study Intervention(s)

The PI will be responsible for determining whether an AE is expected or unexpected in relation to the study procedures and intervention(s) (as applicable).

For device studies: An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described in the clinical protocol, device manual, investigator's brochure, the package insert(s), the IRB application, or the informed consent document. Expectedness is recorded for both study procedures and interventions.

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

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

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

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

The PI will record all reportable events with start dates occurring any time after the administration of the study drug and for up to 30 days after the date of the last dose of study drug. At each study visit, before and after each interventional session, the investigator will inquire about the occurrence of AE/SAEs since the last contact. Events will be followed for outcome information until resolution, stabilization, or completion of study participation.

14.5 Reporting AEs and SAEs

The investigator will immediately report to the coordinating site PI any SAE, whether or not considered study intervention-related, including those listed in the protocol and must include an assessment of whether there is a reasonable possibility that the study intervention caused the event. Study endpoints that are SAEs (e.g., all-cause mortality) must be reported in accordance with the protocol unless there is evidence suggesting a causal relationship between the study intervention and the event (e.g., death from anaphylaxis). In that case, the investigator must immediately report the event to the sponsor.

All SAEs will be followed until satisfactory resolution or until the site investigator deems the event to be chronic or the subject is stable.

14.6 Unanticipated Problems

An unanticipated problem (UP), as defined by the DHHS Office for Human Research Protection (OHRP), is any incident, experience, or outcome that meets all of the following criteria:

- The incidence, experience, or outcome is unexpected given the research procedures described in protocol-related documents (e.g., the study protocol, the informed consent documents, the Investigator's Drug Brochure) and the characteristics of the subject population being studied. An event may be considered unexpected if it exceeds the nature, severity, or frequency described in the study-related documents, Investigator's Device Brochure, product labeling, or package insert.
- The incidence, experience, or outcome is related or probably related to participation in the research study. "Probably related" means the incidence, experience, or outcome is more likely than not to be caused by the research study procedures.
- The occurrence of the incidence, experience, or outcome suggests that the research places subjects or others at a greater risk of harm (physical, psychological, economic, or social) than was previously known or recognized.

The investigator will report UPs to the reviewing IRB. The UP report will include the following information:

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

Report UPs within the timeframe(s) specified by the IRB(s) of record.

14.7 Unanticipated Adverse Device Effect

An unanticipated adverse device effect means any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects (21 CFR 812.3(s)).

An investigator shall submit to the reviewing Institutional Review Board (IRB) a report of any unanticipated adverse device effect occurring during an investigation as soon as possible, but in no event later than 5 working days after the investigator first learns of the effect.

14.8 Incidental Findings

MR image clinical abnormality

If there are any clinically relevant adventitious findings from the baseline or follow-up visits, subjects will be informed; however subjects may not be informed of findings of questionable significance. Participants will be contacted directly (either on the phone or in person) if the results of an imaging study are clinically significant. The clinician will confer with the subject about whether they would like their primary care provider to be notified about any clinically significant findings and contact them if desired.

14.9 Study Monitoring

The Principal Investigators (PIs) will be responsible for ensuring participants' safety on a daily basis.

In addition, the PI, Co-Is and core research staff will meet every two months to monitor study progress and any data and safety issues will be reviewed.

All unanticipated problems and complications will be discussed during regular staff meetings (attended by PI, supervisors, and coordinators) during which a plan of action will be formulated and implemented.

14.10 Study Stopping Rules

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to the investigators. If the study is prematurely terminated or suspended, the PI will promptly inform the IRB and will provide the reason(s) for the termination or suspension.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to subjects
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable

Study may resume once concerns about safety, protocol compliance, data quality are addressed and satisfy the applicable federal and institutional regulatory authorities.

15.0 STUDY FEASIBILITY

15.1 Economic Burden to Subjects

Subjects will not have to pay for study procedures.

15.2 Feasibility of Recruiting the Required Number of Subjects

Recruitment sources: Participants may be recruited by several methods. Major sources are summarized below:

1) Physician Referrals (e.g. from a Memory Assessment Clinic [University of Wisconsin (UW) Hospital and Clinics' Memory Clinics and the Memory Disorder's Clinic in Neurology at Froedtert and the Medical College of WI, Middleton VA Hospital Madison, Froedtert Hospital, MCW Memory Disorders Clinics and the Geriatric Psychiatry Clinic]; the Wisconsin Alzheimer's Institute (WAI) Diagnostic Clinic Network.
2) Participants from the community (through advertisements, brochures, educational /outreach events, etc.)
3) From existing large studies such as the Wisconsin Alzheimer's Disease Research Center clinical core. In addition, we will approach participants who have provided permission to be contacted for future research as part of their participation in Wisconsin ADRC-affiliated studies.

4) WI ADRC Recruitment Registry (REGGIE, 2011-0772) and the Alzheimer's Association Trial Match website.

To maximize retention, study personnel may travel to participants' homes to perform interventions.

16.0 MULTISITE RESEARCH CONSIDERATIONS

16.1 Single IRB (sIRB) Communication Plan

Study Team Communication Plan

Study initiation conference calls will include a presentation by the reliance site's PI to inform all sites about the reliance arrangement as well as the review processes and reporting requirements of the Reviewing IRB

Dr. Granadillo or his delegate will be responsible for ensuring ongoing communication with all participating study teams via teleconferences and regular emails throughout the study. Key communication points will occur to:

- Disseminate IRB determinations and IRB-approved documents
- Educate study teams regarding the approved study and amendments to the study
- Alert study teams to problems that may affect the conduct of the study or the rights and welfare of research participants, such as unanticipated problems and serious noncompliance
- Inform study teams of any changes in study status (e.g., temporary suspensions of recruitment) or new information
- Facilitate submissions to the Reviewing IRB, including:
    - Inclusion of site-specific requirements in consent documents
    - Identification of any variability in study implementation across sites that must be communicated to the Reviewing IRB
    - Collection of information from participating sites to include in continuing review reports to the Reviewing IRB
    - Site-specific amendments
    - Personnel updates (as required by the Reviewing IRB)
    - Reportable events (e.g., noncompliance, unanticipated problems)
    - Closure reports
- Ensure revisions to applicable conflict of interest management plans are provided to the Reviewing IRB

17.0 REFERENCES

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