Effect of modulating gamma oscillations by transcranial alternating current stimulation on brain structure and function in humans

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PART B
STUDY DESCRIPTION

TITLE OF PROTOCOL	**Effect of modulating gamma oscillations by transcranial alternating current stimulation on brain structure and function in humans**
Principal Investigator	**Emiliano Santarnecchi, PhD**

B1. PURPOSE OF PROTOCOL

Thanks to advances in public health and medicine, the life expectancy of the world population continues to lengthen. For the first time in history there are more people 65 and older than younger than 5. While longer lifespan is a unique opportunity for society to benefit from the wisdom and experience of the elderly, aging is however also the greatest risk factor for neurodegenerative disorders and cognitive decline. In addition, a growing number of potential insults, including for example repeated minor traumatic brain injury, are being linked to neurodegenerative processes. A fundamental neurobiological substrate of cognitive decline and neurodegeneration appears to involve alteration of neuroinflammatory processes with associated deposition of aberrant proteins, such as amyloid or tau. Recent pre-clinical work from MIT's Li-Huei Tsai, Ed Boyden and collaborators (Iaccarino et al., 2016) reveals that induction of gamma oscillations in mice can modulate activity of microglia, modify inflammatory brain processes, and lead to clearance of amyloid and tau deposition. Translation of such findings to humans could have transformative impact, with relevance not only for a number of brain diseases, but also more broadly for human cognition maintenance and enhancement across the lifespan.

Here we propose a first-to-human translation of the preclinical data on the effect of induction of gamma oscillations on brain structure and function. We chose to focus on Alzheimer's Disease (AD) because it is the leading cause of dementia, affecting over 5 million people in the United States alone, and over 30 million worldwide (Hebert, Weuve, Scherr, & Evans, 2013). AD was also the sixth leading cause of death in the US in 2013, and may be a major factor in up to half a million more (James et al., 2014). As the population ages, the prevalence of AD is expected to increase significantly in the future, with estimates suggesting that 7.1 million Americans may suffer from the disease by 2025, as many as 15 million by 2050. Despite this enormous disease burden, therapeutic options are very limited. Specifically, while there are pharmacologic interventions that transiently improve cognitive function, there are no treatments that alter disease progression. As such, the development of a disease-modifying intervention would be of great clinical significance.

Our central hypothesis is that 4 weeks of daily sessions of 40 Hz tACS will modulate microglia activation and significantly decrease cerebral beta-amyloid and p-tau depositions. This will be correlated with improvement on electrophysiological and neuroimaging measures of brain function, and on cognitive testing.

Specific Aim #1: To assess the effect of 20 1-hour long sessions of 40 Hz tACS stimulation on microglia activation, amyloid deposition and tau deposition in the brain as measured by PET imaging in patients with AD.
We hypothesize that 40 Hz tACS will modulate microglia activity resulting in a significant decrease in amyloid and tau burden.

Specific Aim #2: To assess the safety of 20 sessions of 40 Hz tACS stimulation in patients with AD.
We hypothesize that tACS will be well-tolerated by all subjects, without any significant side effects.

Specific Aim #3: To assess the effect of 20 sessions of 40 Hz tACS stimulation on brain connectivity (as

assessed by EEG and MRI) and cognitive function (as assessed by neuropsychological testing) in patients with AD.
We hypothesize that 40 Hz tACS will result in an increase of high-frequency (gamma) EEG activity and brain network connectivity on EEG and rsfMRI, and that these changes will be associated with an improvement in memory and general cognitive function.

Exploratory Aim 1: As an exploratory aim, we will analyze the impact of APOE (Apoliprotein E) and BDNF (Brain Derived Neurotrophic Factor) polymorphisms on individual response to tACS(i.e. change in amyloid level and/or gamma EEG spectral power), as measured via EEG and cognitive testing. Such information will be derived via saliva samples.

Exploratory Aim 2: The response to non-invasive brain stimulation might be related to intrinsic brain properties such as brain plasticity and connectivity levels (Freitas, Farzan, & Pascual-Leone, 2013). We will collect information on plasticity levels via a combined TMS and EEG recording session; responsiveness to tACS will be assessed via a combined tACS-EEG session.

Exploratory Aim 3: responsiveness to tACS might depend on the amount of endogenous gamma activity characterizing each individual at baseline. To investigate the relationship between individual levels of gamma activity and response to 40 Hz tACS, we will collect EEG activity during the execution of simple cognitive tasks known for eliciting burst of gamma activity in different brain regions in humans.

B2. SIGNIFICANCE AND BACKGROUND FOR THE STUDY

Histopathologically, AD is characterized by diffuse amyloid-β (Aβ) plaques and phosphorylated tau (p-tau) deposition in neurofibrillary tangles, as well as widespread neurodegeneration. Recent PET imaging studies suggest that progressive amyloid deposition can begin up to 20 years before the onset of clinical symptoms and stabilizes around the time that clinical symptoms begin to be prominent (C. R. Jack et al., 2013; C.R. Jack Jr. et al., 2010). Phosphorylated tau accumulates particularly in the meso-temporal lobes even in the absence of amyloid, and spreads outside the temporal lobes linked with amyloid. Neurodegeneration and clinical symptoms seem strongly correlated with the spread of tau (Pontecorvo et al., 2017). Consequently, the evidence suggests that both amyloid and tau play a critical role in AD pathogenesis, and interventions that reliably and safely decrease the intracerebral burden of amyloid or tau could potentially be of marked clinical importance. Alterations in neuroinflammatory cascades seem to play a pivotal role in the pathogenesis of AD in the deposition Aβ and p-tau, and the neurobiological consequences of the plaques and tangles, contributing the hyperexcitability and epileptogenesis, brain circuit disruption, and cognitive decline (Palop & Mucke, 2016; Sanchez et al., 2012).

Normal cerebral activity is composed of oscillatory activity across a wide range of frequencies, with oscillatory activity in the 30-80 Hz range known as “gamma” activity. A consistent finding in patients with AD is a relative attenuation of such faster frequencies (Babiloni et al., 2015), and dysregulation of gamma activity linked to pathologic network hyperexcitability which is also seen in animal models of AD (Verret et al., 2012). More recently, a seminal study found that exogenously-induced 40 Hz gamma oscillations reduce Aβ levels and amyloid plaques, and may also reduce tau levels, as seen in a mouse model of AD (Iaccarino et al., Nature 2016). In subsequent work, the authors have also determined that inductions of gamma activity in presymptomatic AD mice remarkably prevents subsequent neurodegeneration and behavioral deficits, suggesting that gamma induction may represent a novel and powerful therapeutic approach for AD (Li-Huei Tsai, personal communications),

Work from our center has recently shown the possibility of modulating brain oscillatory patterns in AD patients, with changes in brain connectivity in the gamma band (measured with EEG) observed after administration of antiepileptic drugs (Musaeus, Shafi, Santarnecchi, Herman, & Press, 2017). However, drugs-based interventions do not allow for precise targeting of Aβ or p-tau deposition, while the induction of 40Hz oscillations by means of visual and/or auditory stimulations (as those implemented in the aforementioned animal model by Iaccarino et al. 2016) are limited by the nature of the stimulation, i.e. they supposedly affect visual and auditory cortices. PET imaging with different radioactive tracers allows to characterize individual patterns of Aβ and p-tau deposition, which might be present in various cortical sites not necessarily matching the regions of action of sensorial stimulation approaches.

The present proposal will leverage current knowledge on transcranial electrical stimulation to collect proof-of-principle data on an individualized intervention that aims to modulate neuroinflammation and reduce Aβ and p-tau deposition by means of tACS. tACS is a safe, noninvasive technique utilizing low amplitude alternating (sinusoidal) currents to modulate brain activity and cortical rhythms. Specifically, tACS entrains cortical rhythms at the applied frequency during stimulation (Frohlich & McCormick, 2010), and can produce sustained increases at the applied frequency for up to 70 minutes after the end of stimulation (Kasten, Dowsett, & Herrmann, 2016). Notably, gamma-frequency tACS has been used to transiently improve human performance on tasks involving motor performance, working memory and even intelligence ((Santarnecchi et al., 2013; Santarnecchi, Muller, Rossi, Sarkar, Polizzotto, Rossi, & Cohen Kadosh, 2016b; Santarnecchi et al., 2017)). Most importantly, tACS can be targeted to theoretically any cortical brain region, allowing to match the stimulation pattern with individual amyloid and tau burden maps obtained through PET imaging.

Recent studies in the field of non-invasive brain stimulation (NiBS) suggest the feasibility of interacting with brain oscillations by means of tACS, where low intensity (max 2mA) alternating sinusoidal currents are applied via scalp electrodes. Due to the safety (Rossini et al., 2015) and controllability (in terms of

stimulation frequency and the possibility to target almost any cortical region) of the procedure, tACS has been promoted as one of the most promising techniques to modulate the healthy and pathological brain (Tatti, Rossi, Innocenti, Rossi, & Santarnecchi, 2016). Animal work has demonstrated that tACS entrains neurons in widespread cortical areas (Ozen et al., 2010), and emerging experimental evidence shows that the effects of weak electric fields applied on optogenetically-controlled slices of pyramidal cells are constrained by their own endogenous cortical oscillations (Frohlich & McCormick, 2010). Simulations, supported by empirical evidence using EEG, demonstrate that tACS modulates brain oscillatory activity via network resonance, suggesting that a weak stimulation at a resonant frequency could cause large-scale modulation of network activity (Schmidt, Iyengar, Foulser, Boyle, & Frohlich, 2014), and amplify endogenous network oscillations in a frequency-specific manner (Frohlich & McCormick, 2010).

In humans, tACS modulates brain activity, with effects being documented at the behavioral level for sensorimotor (Santarnecchi et al., 2017)(Feurra, Bianco, et al., 2011; Feurra et al., 2013), visual (Kanai, Chaieb, Antal, Walsh, & Paulus, 2008), somatosensory (Feurra, Paulus, Walsh, & Kanai, 2011) and higher-order cognitive domains (Santarnecchi et al., 2013, 2016), with effects lasting for up to 70 minutes after stimulation (Kasten, Dowsett, & Herrmann, 2016). Our team at the Berenson-Allen center for Non-Invasive Brain Stimulation has extensive experience with tACS applications in humans, gathered over multiple funded studies and hundreds of tACS sessions, with no significant adverse effects reported so far.

In particular, stimulation in the gamma band (i.e. 40Hz) on the prefrontal cortex of healthy humans has been shown to induce behavioral effects including an increase of abstract reasoning abilities (Santarnecchi et al., 2013) a cognitive function previously demonstrated as to be linked with fast –gamma— oscillatory activity using EEG (Amidzic, Riehle, Fehr, Wienbruch, & Elbert, 2001)(Herrmann, Frund, & Lenz, 2010). The effect has been shown to be frequency specific and initial evidence support the idea of entrainment of brain spontaneous gamma oscillations as the putative mechanism for such effect. A subsequent study in collaboration with Oxford University has further validated tACS applications for cognitive enhancement, also showing evidence of the effects being limited to the region –and cognitive function- being stimulated (Santarnecchi et al., 2016). Most importantly, the study also showed how individual differences in baseline cognitive performance significantly predict the response to tACS in the gamma band, suggesting the idea of using the response to tACS as a marker of brain reactivity in healthy and pathological conditions.

The possibility of entraining gamma oscillations in humans is not limited to brain regions supporting higher order cognition. A recent investigation by our group has shown how tACS at 60Hz and 80Hz (high-gamma) over the motor cortex is able to modulate visuo-motor performance in healthy participants (Santarnecchi et al., 2017), providing causal evidence of the relevance of gamma-burst previously recorded in the motor cortex during visuo-motor tracking. Additional evidence also suggests the possibility of increasing gamma oscillations in the temporal lobe, with significant long-lasting modifications of ongoing gamma spectral power after stimulation (Santarnecchi et al., under revision, eLife).

This prior work thus demonstrates the feasibility of using tACS to target any cortical region, constituting a significant advantage as compared to other methods for induction of gamma activity such as visual and auditory stimulation (respectively inducing weaker frequency specific responses, also limited to the occipital and temporal lobes of the brain). This becomes even more relevant when targeting amyloid PET-positive brain regions, whose distribution varies across patients.

Moreover, new technologies developed by our center in collaboration with external partners (Neuroelectrics, Barcelona, Spain) might help to further increase the precision of tACS targeting, allowing for individualized stimulation solutions based on modeling of current distribution using structural MRI scans. The first generation of devices for transcranial electrical stimulation only allowed for stimulation protocols including two electrodes, limiting the number of target regions to no more than two.

Moreover, this solution did not allow for careful mapping of individual brain anatomy, and therefore resulted in sub-optimal stimulation patterns. Current approaches for so-called multi-focal stimulation (Ruffini, Fox, Ripolles, Miranda, & Pascual-Leone, 2014) permit stimulation montages based on up to 32 stimulating channels, with the precise stimulation pattern defined by means of modeling of induced electric field based on individual T1-weighted MRI scans. This results in more accurate, individualized montages, which might become crucial when targeting amyloid in AD patients.

Also, genetic testing is increasingly playing a role in the understanding of the etiology of AD. In particular, certain polymorphisms in the gene which codes for the apolipoprotein E (ApoE) have been linked to increased risk of developing AD, specifically with carriers of the ε4 allele (Risacher et al., 2013). Furthermore, there is at least some evidence that ApoE-ε4 may impact response to non-invasive brain stimulation (Peña-Gomez et al., 2012), therefore potentially modulating the effect of tACS.

This study will leverage all this accumulated knowledge by implementing an intervention based on multiple, individualized multifocal tACS stimulation sessions based on individual PET and MRI information in patients with amyloid-positive PET with the hope that this leads to microglia activation and decrease in cerebral amyloid and tau depositions in human patients with AD.This would have immense translational impact, as gamma tACS is an intervention that is portable, does not require expensive hardware, can be widely applied to large numbers of patients with AD, as well as, given its favorable side effect profile, even to patients at earlier stages of the disease who have cerebral amyloid/tau without clinical symptoms.

B3. DESCRIPTION OF RESEARCH PROTOCOL

A. Study Design – Overview, Methods, Procedures

Overview

This is an early phase study of tACS in patients diagnosed with early AD. Individuals who have had amyloid PET imaging and have evidence of cerebral amyloid burden or patients who, in their physician's judgment, have a strong likelihood of cerebral amyloid burden will be recruited. All subjects will receive 20 sessions of active tACS targeted based upon individual tracer uptake on the amyloid PET study.

Study Design

The study will be conducted at BIDMC, at the Berenson-Allen Center for Noninvasive Brain Stimulation and in the BIDMC Clinical Research Center except for the PET scans. The PET imaging will occur at Massachusetts General Hospital (MGH) in the Gordon PET Center and will be submitted as a separate protocol at MGH that describes the pre and post measures of PET/CT.

We aim to enroll 10 individuals with AD with evidence of increased cerebral amyloid burden on amyloid PET imaging. This would allow for a final sample size of 5-6 fully evaluable subjects. Each subject's participation in this study will consist of approximately 31-35 visits: 1 day for consent and screening procedures, 5-7 days of baseline procedures (this includes the PET scans), 20 tACS study visits, and 5-7 days of follow-up assessments. Subjects will undergo baseline cognitive assessment, structural and functional MRI characterization, PET imaging to assess amyloid burden, tau deposition and level of microglia activation (if results of genotyping allow), and resting-state EEG measurement. Additionally, patients will undergo a TMS-EEG and a tACS-EEG recording session to assess brain plasticity levels and identify markers of response to stimulation. All subjects will subsequently undergo 20 sessions of gamma-frequency (40 Hz) tACS. The stimulation sites will be identified on a case-by-case basis taking into account the overall distribution of amyloid- ß as evidenced by the amyloid PET scan. Subjects will take a standardized adverse effect questionnaire before and after each session to demonstrate safety and tolerability (Fertonani et. Al, 2015). At the end of the 20 sessions, subjects will then repeat the baseline assessments over 5-7 visits, including repeat PET imaging to assess for changes in amyloid burden, tau deposition, and microglia activation.

Procedures

Screening Visit (Visits 1)

During the screening visit, participants will provide informed consent and complete the following procedures. Please note the screening schematic below:

- Neurological exam*
- Demographic review*
- Review of medical, psychiatric and medication history, including review and confirmation of diagnosis*
- tACS and TMS safety questionnaires*
- MMSE*
- Pregnancy test for females of child-bearing potential*

- MRI screening questionnaire*
- Pre PBR PET Imaging (microglial PET) Genetics Screening (potential participants with low affinity binding to the ligand will not complete the microglia PET scan– see details in "Methods" below)*
- Review of research radiation exposure over the past 12 months
- Confirmation of amyloid positive PET scan. If a subject does not have a prior amyloid PET scan, this will be completed via the MGH PET imaging protocol. The scan will be read by a neuroradiologist to establish a positive or negative read.*
- Inclusion/Exclusion criteria review – including potential exclusions for the MGH PET imaging protocol*
- The Clinical Dementia Rating (CDR) scale to assess severity of dementia
- Alzheimer's Disease Assessment Scale Cognitive Subscale (ADAS-Cog)
- Baseline Clinical Global Impression of Change (CGIC)

All criteria needed to determine study eligibility will be completed during the screening period as indicated by the asterisks above (*).

Baseline Visits

The baseline visits will occur over 5-7 days. It is possible that screening activities listed above that are not necessary for inclusion (e.g. CGIC), will be completed at one of the baseline visits. The remainder of the activities will be planned according to scheduling, resources, timing and subject tolerability. It is possible that a baseline activity will happen on the first day of stimulation prior to the tACS (e.g. a questionnaire). If a subject is having difficulty completing all of the baseline testing, it is possible that the TMS-EEG and/or the tACS-EEG visits will be stopped or not completed based on the participant's ability to tolerate the visits.

- PET Imaging – PET images will be obtained via the MGH PET/CT protocol to assess amyloid burden, tau deposition, and microglia activation (if possible, based on results from genotyping) – details of scheduling and order of PET scans are detailed further below in "Methods" section.
- National Alzheimer's Coordinating Center's Uniform Data Set (NACC-UDS) questionnaires: Activities of Daily Living Inventory (ADL); Geriatric Depression Scale (GDS); Functional Assessment Questionnaire (FAQ)
- Cognitive testing:
    - National Alzheimer's Coordinating Center's Uniform Data Set (NACC-UDS) battery that includes the MoCA, Number Span Forward & Backward, Trail Making Test, Figure Copy, Story Recall, Digit Symbol, and Multilingual Naming Test, Verbal Fluency, Category Fluency. Only portions of this set may be completed, although the MoCA will be done in all cases as this will serve as the baseline cognitive assessment for monitoring cognition throughout the study.
    - Brief estimate of intelligence using the Wechsler Test of Adult Reading (WTAR)
    - Rey Auditory Verbal Learning test (RAVLT) as measure of learning and memory

  Only portions of this testing may be completed, based on participant tolerability although the MoCA will serve as the baseline cognitive assessment for monitoring cognition throughout the study, therefore will be done in all cases.
- Modified Edinburgh Questionnaire to determine handedness
- Saliva for DNA and tau (this may be collected at any visit)

  - Three saliva samples will be taken:
    - One sample for tau protein
    - Two optional samples for BDNF and APOE genotyping (this may be collected at a subsequent visit if the subject has trouble producing additional saliva following the first collection)
- fMRI - Patients who have had an fMRI in another study in the Berenson-Allen Center within 6 months (pending the quality of the images) may not have to repeat this scan
- Lumbar puncture** (optional)
- Blood Draw**
- TMS-EEG plasticity assessment
- tACS-EEG with cognitive tasks

**Blood will be sent, de-identified to Boston Children's Hospital, Molecular Genetics Core Facility (Boston, MA) for screening of the Ala147Thr polymorphism needed for the microglial PET scan. If the participant agrees to an LP, blood and CSF will be sent for real-time analysis (e.g. Labcorp) of CBC, glucose, and protein to assess for any incidental LP finding (e.g. indicators that there is concern for an inflammatory process). CSF and blood will also be processed and stored for analysis of AD biomarkers at the end of the study by an outside laboratory, Quanterix (Lexington, MA). If the participant does not agree to the LP, the blood will only be analyzed for AD biomarkers at the end of the study.

If abnormal results are found from the LP, a neurologist on the study in the Berenson-Allen Center will review the results. The neurologist will determine if the finding is in need of clinical follow-up. If this is the case, the neurologist will speak with the participant, will provide a letter describing the findings and need for follow-up and will be available to speak with the participant's provider if they participant agrees and gives permission to do so.

tACS visits (treatment visits 1-20)
The tACS study visits will be conducted in the Berenson-Allen Center for Noninvasive Brain Stimulation at BIDMC or in the Clinical Research Center. Participants will undergo 20 days (weekdays) of tACS. Participants will be allowed to miss up to 3 visits for a total of 17 days of tACS. Additional sessions will be added on to reach 17-20 visits if it is within a reasonable timeframe as determined by the investigator. Each session will consist of the following:

- Review of tACS side effects and adverse events will be completed daily before and after stimulation. An assessment of any changes in medication or medical history will be assessed on a daily basis.
- Set up for EEG and tACS which includes cleaning the scalp with alcohol, placing a cap with electrodes on the participant's head and applying gel underneath electrodes
- Three minutes of eyes-closed and eyes-open resting state EEG
- 1 hour of 40Hz tACS stimulation to targeted brain regions
  - Additionally, EEG will be recorded throughout the tACS stimulation
- Cognitive assessment (MoCA) will be completed daily to monitor any cognitive changes. If the score drops by 4 points or more, the covering neurologist will be alerted to assess the participant further. The following day, the patients will repeat a MoCA and if the score has not improved they will be reassessed by the neurologist and the participant will not receive stimulation.

- Participant tolerance:
    - Subjects will be queried each day about their experience and how they are doing. If participants or their family members express that the participant is having difficulty, study staff will work with them to reduce any burden if possible (e.g. arranging reliable transportation, assuring that they have a snack if needed…..).
    - A "Participant Experience Assessment" will be completed with the subject and/or the study partner at the end of each week to monitor participant burden.

Follow Up Visits
The follow-up visits will occur over 5-7 days within approximately 3 weeks after the completion of the tACS study visits. If any of the assessments were not completed due to the subject's ability to tolerate the procedures (e.g. TMS-EEG), then these will not be done at follow-up:

- Cognitive Evaluation that includes the MMSE and any of the assessments collected at baseline (e.g. ADAS-Cog, UDS, CGIC).
- Repeat baseline UDS questionnaires
- PET Imaging – Follow up PET images will be obtained to assess the change in microglia activation, if assessed at baseline, and change in amyloid and tau burden.
- fMRI
- Lumbar puncture (optional – and only completed if done at baseline)
- Blood Draw
- TMS-EEG plasticity assessment
- tACS-EEG with task recording
- Adverse event review and follow-up
- Review of medications and changes in medical history

If participants have plasticity measures or neuropsychological testing from a prior study in the Berenson-Allen Center, those may be compared to the measures obtained in this study and/or used in place of the testing in this study.

1-month Follow-up
The participant and study partner will return to BIDMC approximately 1 month after completing the follow-up procedures. The visit will last approximately 1.5 hours. During the visit the following measures will be collected:

- EEG
- MoCA completed with study participant
- CGIC will be completed with study participant and study partner
- Activities of Daily Living Inventory (ADL) will be completed with the study partner

3-month Telephone Follow-up
The 3 month follow-up assessment will be completed over the telephone. The following measures will be collected:

- Telephone version of MoCA completed with study participant

- CGIC to be completed with study participant and study partner
- Activities of Daily Living Inventory (ADL)- (the number of questions in this may be reduced)

Study Partner Role

All participants will be required to have a study partner who will be asked to volunteer to assist the participant throughout the study. This role includes joining in the informed consent process, assistance with providing information about the subject's medical history, medications, and potential side effects. This person would be interviewed for the Clinical Dementia rating scale at the beginning and the end of the study. They may be asked to assist with arranging and keeping track of study visits and transportation. They will be asked to communicate any difficulties or adverse effects that the subject may be experiencing during participation. The study partner has the right to decline or stop participation at any time. If so, the participant will be asked to choose a new study partner for the remainder of the study. A consent script has been developed for the Study Partner Role.

Methods

PET/CT

All PET/CT procedures will take place at MGH under an MGH submitted and approved protocol that is specific to this study. Participants will be informed that participating in the PET imaging is a required part of the overall study during consent. They will be provided with information about the number of visits, basic procedures and risks during consent for this protocol to allow them to make a decision about proceeding. A separate consent will be obtained at MGH by a study MD knowledgeable about the PET scans being conducted. Pre-screening for the PET imaging will be completed through this protocol in order to determine if the participant is able to complete the microglia PET scan.

Pre- PBR PET (microglial PET) DNA Screening

All subjects will undergo genotyping for the Ala147Thr polymorphism in the translocator protein (TSPO) gene (rs6971) as part of the screening procedures. This polymorphism predicts [11C]PBR28 binding affinity (with Ala/Ala, Ala/Thr and Thr/Thr predicting high, mixed or low affinity binding). The [11C]PBR28 will be used in one of the PET imaging procedures to be conducted within this protocol. The results of the DNA will be used to pre-screen participants who are low-affinity binding (Thr/Thr), therefore those participants will not complete the microglia PET scan at MGH. It is estimated that 9% of the population has this low affinity binding genotype. The blood samples will be de-identified and sent to Boston Children's Hospital, Molecular Genetics Core Facility (Boston, MA) to analysis.

Pre PET/CT Scans (Study Visits #1-3):

The PiB (amyloid), T807 (tau) and PBR (microglial) PET/CT scans do not have to be completed in any specific order for the baseline unless amyloid status is unknown. If that is the case, the PiB scan will be completed first in a single visit and the participant will not proceed until the scan has been read by a neuroradiologist (from either MGH or BIDMC) prior to proceeding per inclusion criteria. If the participant is amyloid negative, they will not proceed in the study. If the participant proceeds, the T807 and PBR scans will be completed over one or two visits depending upon subject preference and tolerability. If the amyloid status is known, the three scans will be scheduled according to subject preference and tolerability – e.g. an attempt will be made to complete 2 scans in one day with the third on another or to complete the scans in three separate visits. All pre-PET/CT scans will be completed within 2-3 weeks prior to proceeding to the tACS intervention.

Post PET/CT Scans (Study Visits #4-6):
All 3 post-treatment PET/CT (or 2 scans, if the participant does not complete the microglia scan based on the results of the genotyping) scans will be completed within 3 weeks following the final tACS study visit. If the participant was able to tolerate having two scans in a single visit pre-intervention, then this will be attempted again. If the participant cannot tolerate undergoing two scans in one day, a 3rd post visit will be completed. The order of the scans does not matter if they are completed on separate days.

Amyloid PET imaging to assess Aβ burden is not routinely collected clinically due to lack of insurance coverage, but is of possible clinical relevance in the diagnosis and treatment of the participant. The scan will be read by a nuclear medicine physician and a report will be prepared. If the participant has not had a prior amyloid PET, they will be informed if the scan was positive or negative as part of the inclusion in to the study. The investigator will talk to the subject about their amyloid imaging to assess if the subject is interested in sharing the results of the PET scan with a provider so that the results can be reviewed with them in the context of their clinical situation. If there is interest in sharing the results, they will be given to the participant's designated provider or a provider will be identified for them through a referral to the cognitive neurology unit at BIDMC. The aim is to provide a method to share results with the participant within the context of their clinical situation. The results of the Tau and microglial PET scans will not be shared with the participant as they are collected for research only and there is currently no evidence that the results of these scans would contribute to the clinical care of the participant. Results of the amyloid PET scans outside of the initial scan will not be shared with the participants as there is no evidence that these results would be clinically relevant either.

Cognitive Measures

Alzheimer's Disease Assessment Scale (ADAS-Cog):
The ADAS-Cog is a standardized neuropsychological assessment that measures the severity of symptoms of Alzheimer's Disease in 11 different domains. The tasks assess the domains of language, praxis, memory, attention and executive function.

Alzheimer's Disease Cooperative Group Study Clinical Global Impression of Change (CGIC):
The CGIC is a way of assessing the AD subject's global change from baseline in a clinical trial. There is a semi-structured baseline interview of the subject and family member or support person. This interview includes questions surrounding the subject's memory, behavior, thought content, and functioning, to name a few. In subsequent visits, the AD subject and family members are interviewed again and the clinician makes an assessment of the subject's change on a Likert-type scale (e.g. improvement, no change, worsening) from baseline.

National Alzheimer's Coordinating Center's Uniform Data Set (NACC-UDS):
The NACC-UDDS is a neuropsychological test battery from the Unified Data Set of the Alzheimer's Disease Centers program of the National Institute of Aging. The battery consists of brief measures of attention, processing speed, executive function, episodic memory and language. The battery includes the following assessments: MoCA, Number Span Forward & Backward, Trail Making Test, Figure Copy, Story Recall, Digit Symbol, and Multilingual Naming Test, Verbal Fluency, Category Fluency.

Cognitive Dementia Rating Scale (CDR):
The CDR is a five points rating scale evaluating the severity of dementia. Six domains are assessed by the clinician using a structured interview of the patient and a family member/caretaker: memory,

orientation, judgment and problem solving, community affairs, home and hobbies, and personal care. These assessments are based upon the subject's cognitive ability to perform in each realm.

Minimental State Exam (MMSE):
The MMSE is a brief, widely used valid and reliable assessment of cognitive impairment. This 30 point questionnaire is used to screen for and estimate severity of cognitive impairment in addition to being used to follow the course of cognitive change over time. The MMSE assesses orientation, attention and calculation, recall, language and repetition and ability to follow complex commands.

Montreal Cognitive Assessment (MoCA):
The MoCA is a widely used 30-point test. It assesses multiple domains including short-term memory recall, visuospatial ability, executive function, attention, concentration, working memory, and orientation. The telephone version of this tool will be utilized for phone follow-up (T-MoCA). The T-MoCA is the same as the eMoCA with the removal of the pencil/paper and visual portions of the assessment. The test takes approximately 10 minutes to complete.

Rey Auditory Verbal Learning Test (RAVLT):
The RAVLT consists of a list of 15 unrelated words repeated after 5 trials and are asked to repeat. Another list of 15 unrelated words is given and the participant is then asked to repeat the original list of words immediately and then after 30 minutes. This is an assessment of short-term auditory-verbal memory, rate of learning, learning strategies, interference, presence of confabulation of confusion in memory process, retention and differences between learning and retrieval.

Wechsler Test of Adult Reading (WTAR) – ONLY completed at baseline:
The WTAR is a neuropsychological assessment used to assess a pre-morbid level of intellectual functioning prior to the onset of an illness or disease. It consists of a series of irregularly spelled words that are presented to the subject while prompting them to pronounce the word.

Additional Measurement Instruments:

Handedness Questionnaire – ONLY completed at baseline:
The handedness questionnaire is an assessment of hand dominance, based on the Edinburgh Handedness Inventory (Oldfield, 1971).

National Alzheimer's Coordinating Center's Uniform Data Set (NACC-UDS) questionnaires:
The following questionnaires will be collected: Activities of Daily Living Inventory (ADL); Geriatric Depression Scale (GDS); Functional Assessment Questionnaire (FAQ) - Please see attached.

MRI
MRI will be performed on BIDMC's 3T GE MR750 MRI Scanner. A high resolution T1-weighted structural scan will be obtained from the scanner. The MRI will be used in combination with the PET images to define the stimulation targets. Additional standard MR protocols to assess resting-state functional connectivity, cortical metabolism, or white-matter integrity, may be included if time allows.
All MRI imaging will be conducted at the Beth Israel Deaconess Center Medical (East campus). The subject will be brought into the scanner room and instructed to lie down on a foam-padded table that can slide into the scanner. Subjects will be handed the emergency button which they can squeeze at

any point during the procedure to inform the investigators if they have a question, are feeling uncomfortable, or are in distress. The subject's head will be carefully positioned and foam-padded cushions will be placed on either side of their head in order to prevent movement during the scanning session. The subject will receive earplugs to wear during the entire scanning session in order to minimize the noise of the scanning machine. The subject will then undergo a series of MRI scans of the brain. Structural images will be acquired followed by resting-state functional MRI scans. Total scan time will be about 60 min.

The MRI done in this study is for research purposes only. It will not be read by a radiologist. If there are incidental findings noted on the MRI, the subject will be notified and advised to see their primary care provider for a diagnostic MRI.

For safety reasons, participant whose abdomen, shoulder, or hip circumference is greater than 180cm may need to be excluded from the study.

TMS – EEG: Brain plasticity and responsiveness to stimulation
The TMS-EEG measures will be collected at baseline and then again following the 20 day tACS intervention to assess for changes in neural plasticity. Subjects will be set up in a chair with an EEG cap and with EMG electrodes placed on the right hand for collection of motor evoked potentials (MEPs) during stimulation over the left primary motor cortex (M1). The EEG cap and EMG electrodes will remain in place throughout the TMS session. EEG will be recorded throughout the session. Participants will be provided ear protection to be worn throughout the session. TMS specific adverse effect review will be reviewed prior to and at the end of the session.

- **Baseline Resting State EEG and artifact recording:**
  Baseline resting state EEG and artifact recordings will be obtained with the eyes open for five minutes and then eyes closed for five minutes. Subjects will be asked to briefly move their eyes, clench their jaws, and tense their foreheads so that the EEG artifacts associated with these movements can be recorded and similar artifacts removed from the remaining EEG recordings.

- **Assessment of Motor Threshold:**
  Resting motor threshold (RMT) will be determined by applying single pulses to M1. RMT will be defined as the minimum stimulus intensity that produces a motor evoked potential (MEP) of at least 50 μV in the hand muscles in at least 5 of 10 trials. MEPs will be measured by electromyography (EMG) during relaxation of the tested muscles. Determination of RMT will be used to guide intensity to be used for single pulses as well as paired-pulses and for stimulation intensity iTBS protocol. The optimal position for obtaining MEPs will be identified at the beginning of the assessment of RMT.

- **Pre and Post Single and Paired Pulse TMS-MEP Assessments:**
  Baseline cortical reactivity will be assessed by applying single pulses of TMS to up to six different non-motor cortical regions. Paired-pulse TMS will be applied to the prefrontal target. Cortical reactivity will be assessed via EEG measures (TMS-evoked potentials - TEPs). Additionally, cortico-motor reactivity will be assessed at M1 prior to and following the iTBS stimulation by measuring peak-to-peak amplitude of MEPs induced in the hand muscles in response to single pulse TMS as measured by EMG. TMS intensity will be set at 120% of each individual's resting motor threshold for single and paired pulses.

Following the pre-assessments of non-motor cortical targets, batches of single pulse TMS to M1 will be recorded prior to iTBS and used as baseline. Following iTBS, batches of MEPs will be measured at 5-10 minute intervals for up to one hour. An index of modulation of motor cortical excitability will be calculated as the percentage change of mean MEP amplitude, post-TMS relative to pre-TMS, with positive values (MEP amplitude increase) reflecting facilitation of cortical excitability by TMS, and negative values (MEP amplitude decrease) representing suppression.

- **Repetitive TMS – iTBS Protocol:**
  Intermittent/continuous theta burst stimulation will be delivered using a figure-of-8 coil. Prior to stimulation, the active motor threshold (AMT) will be identified. AMT will be defined as the minimum stimulus intensity that produces an MEP of at least 200 μV that is followed by a cortical silent period (absence of background EMG activity) in at least 50% of 10 trials. MEPs will be assessed during isometric contraction of the tested muscles, at approximately 20% of maximum voluntary contraction. Stimulation intensity will be set at 80% of AMT. At times, AMT can be difficult to determine. If this is the case, stimulation intensity will be set at 70% of RMT, which is within 5% of AMT. iTBS will be applied to the M1 location identified with RMT assessment and consists of 2-second trains, each with bursts of 3 TMS pulses at 50Hz repeated at intervals of 200ms, with 8s pauses between trains (600 total pulses).

tACS-EEG registration

The possibility to induce gamma oscillations is dependent on several neurophysiological genetics and neuroimaging variables, all contributing to the inter-individual variability in gamma-induction via tACS observed in previous studies (Santarnecchi et al. 2013, 2015, 2017). The present protocol includes careful monitoring of all these variables, via e.g. baseline EEG measurements, genetic data, structural and functional MRI data. Additionally, in order to estimate the likelihood of induction of gamma in each participant, a shorter tACS-EEG session will be carried out to see how each participant's brain responds to brief tACS bursts. The immediate response (e.g. increase/decrease of gamma power) after short tACS stimulation blocks (up to 20 minutes) will be collected via EEG recording before/during/after tACS. Such response will be then used to predict the response to the full tACS intervention (20 daily 1 hour sessions). Specifically, tACS will be applied to up to 4 brain regions for each brain hemisphere, including a Sham stimulation block. Stimulation intensity will not exceed limits suggested by tCS safety guidelines, equal to 2mA per stimulation electrode and 4mA total injected current across all stimulating electrodes. At the beginning of the visit (i.e. before any of the tACS stimulation blocks), the individual threshold for perception of phosphenes induced by tACS will be also estimated. Moreover, in order to collect information about brain's ability to evoke gamma activity in response to stimuli different than tACS, we will monitor the amount of gamma activity induced by brief cognitive tasks delivered using a regular desktop PC connected to the EEG system. Participants will be asked to observe images on the screen or perform basic operations, e.g. press a button when a stimulus appear, which have been shown to elicit a gamma response in humans in previous studies. The tasks will take no more than 20 minutes total. Importantly, the daily exposure to tACS could induce a beneficial change in the way participant's brain respond to tACS (i.e. increase in the individual responsiveness to gamma stimulation), which could increase the effectiveness of repeated tACS treatment cycles. To quantify this change, the same tACS-EEG recording session described above will be repeated at the end of the protocol.
tACS can elicit phosphenes - a sensation of light caused by excitation of the retina by mechanical or electrical means rather than by light. Participant's individual threshold for phosphenes detection will be assessed at the beginning of the tACS-EEG session. We anticipate that a low percent of participants will experience this sensation (10%).

Saliva Sample for DNA and Tau

Three saliva samples will be collected. One sample for tau protein and two optional samples for APOE and BDNF genotyping. Saliva samples will be collected in the Clinical Research Center and/or

the Berenson-Allen Center will be de-identified using a random number, then batch shipped. The samples for APOE and BDNF will be sent to an outside laboratory, Boston Children's Hospital, Molecular Genetics Core Facility (Boston, MA), for analysis. The tau samples will be analyzed at a lab at BIDMC.

Lumbar Puncture (optional) – CSF Biomarkers

The lumbar puncture will be completed at baseline and then following the tACS intervention if the participant is in agreement. Each LP procedure will take about 2-3 hours, and will be performed by the study Neurologist in the Cognitive Neurology Unit or in the Clinical Research Center. During the LP, participants are placed in a left lateral position, with the back flexed, and knees are drawn up towards the chest. The lumbar region of the back will be cleaned with betadine, twice. Lidocaine 1% will be injected into the subcutaneous area between the L3-4 or L4-5 spinous process. Once the area is numb, an LP needle will be placed and CSF will be collected. To clear any blood from minor trauma associated with needle insertion, the first 1-2 ml of CSF are discarded (or more if needed) to eliminate blood, and then 10-20 ml (2-4 teaspoons) of CSF will be collected. CSF will be processed by the Clinical Research Center laboratory. Approximately half will be sent to LabCorp for processing, and the remainder will be frozen, and later batch-shipped, de-identified, to an outside laboratory, Quanterix (Lexington, MA), for processing or stored for future testing. Participants will be asked to lie flat for 1-1/2 to 2 hours following the LP and given discharge instructions and a phone number to call if they have any concerns. The day after the LP the participants will be contacted by phone to see how they are feeling.

The risks associated with a lumbar puncture are primarily related to the development of a spinal headache. Fortunately, the risk is reduced in the elderly and will be further reduced by the use of a small gauge spinal needle. The benefits for the LP are that it will allow for determination of changes in tau level that cannot be measured in any other way. Given the critical role for tau in Alzheimer's disease, a reduction of the elevated tau levels in the CSF after the intervention would be a strong signal of target engagement and efficacy. The LP will also be used to correlate the presence of amyloid in CSF with the presence displayed on the PET scans. Patients will be informed of the risk/benefit ratio and reminded that it is an optional procedure. Analyses of CSF samples will include multiple markers, such as Abeta42, Abeta40, total tau, phospho-tau181, cytokines, YKL-40, and BDNF. Assays will be performed by the central biomarker laboratory. CSF samples will also be frozen and stored for future analysis of putative biomarkers. Local analysis of CSF will be done at LabCorp and will include cell count, glucose, and protein.

Blood Draw

The blood draw will be completed at baseline and follow-up for testing of the same biomarkers as tested in the CSF, such as, Abeta42, Abeta40, total tau, phospho-tau181, cytokines, YKL-40, and BDNF. These results will be used to correlate with the CSF results. In addition, if a patient opts not to undergo the lumbar puncture, the blood tests for biomarkers will provide valuable information. Blood draws will be collected in the Cognitive Neurology Unit or in the Clinical Research Center. A maximum of 20mL (approximately 10mL for the biomarkers and 10mL for CBC, glucose, and protein) will be collected and processed by the Clinical Research Center laboratory. If the participant has an LP, blood will be sent to LabCorp for a CBC with differential, glucose, and protein to correlate with the LP analysis for any abnormalities/possible incidental findings. The remainder will be frozen, and later batch-shipped to an outside laboratory, Quanterix (Lexington, MA), for processing. Assays will be performed by the central biomarker laboratory. Blood samples will also be frozen and stored for future analysis of putative biomarkers.

tACS (Starstim Device)

tACS will be administered using a 32-channel device. The tACS will be applied daily for 60 minutes for 20 consecutive weekdays. The Starstim is also capable of recording EEG. Five minutes of eyes-

closed and eyes-open resting state EEG will also be recorded before and after each stimulation session, to assess the degree of entrainment of gamma oscillations during each tACS stimulation session.

tACS involves the administration of low-amplitude (< 2mA) sinusoidal electrical currents via scalp electrodes. Current will be applied in the gamma frequency (40 Hz) range based on individual amyloid deposits noted on the amyloid PET scan. Although tACS is usually administered via bipolar montages using two large electrodes, such montages have poor spatial specificity. Our group has been at the forefront of efforts using multifocal (multielectrode) montages that can deliver higher amplitude and more spatially specific stimulation patterns (Ruffini, Fox, Ripolles, Miranda, & Pascual-Leone, 2014). Consequently, stimulation will be applied to the target region with maximum amyloid levels using individualized multifocal (multielectrode) montages to maximize the induced electrical current in the target region. The topographic map created from the amyloid PET scan will be overlaid on participant's MRI scan in order to determine the distribution of what ideal current should be used for stimulation, The stimulation montage will be optimized so that the induced electric field is higher on the target regions and null on the rest of the brain. The optimization procedure will be done using the Stimweaver algorithm to calculate electrode configuration (Ruffini, Fox, Ripolles, Miranda, & Pascual-Leone 2014).

Stimulation will be slowly ramped up/down at the beginning/end of each stimulation session to minimize skin sensation. Participants will be queried each day at the end of the visit to see if they experienced phosphenes. They will be informed that this may happen and will be informed that they are free to ask to stop participation if the phosphenes bother them.

EEG
At the 1-month follow-up visit, resting state EEG will be obtained with the eyes open for five minutes and then eyes closed for five minutes. Subjects will be asked to briefly move their eyes, clench their jaws, and tense their foreheads so that the EEG artifacts associated with these movements can be recorded and similar artifacts removed.

B. Statistical Considerations

Sample Size Justification: Given the intrinsic difficulties in the care of AD patients and the commitment required by the multi-day tACS intervention, we expect an attrition rate higher than 20%, potentially close to 30%. We will compare individual changes in microglial activation and amyloid and tau deposition. In order to get valuable pilot data about changes in PET markers, we will enroll 10 patients, aiming for a final sample of 5/6 fully characterized participants who will complete the tACS intervention as well as pre-post multimodal evaluation.

Data Analysis:
Changes in EEG metrics (e.g. gamma spectral power), clinical and cognitive scores, as well as brain plasticity levels, will be measured using paired-sample t-test statistics, by applying a p.value <0.05. Details about computation EEG-based measures are described below.

TMS-EEG and tACS-EEG analysis - Specifically, the TEPs will be studied using traditionally employed metrics such as the absolute magnitude and time-to-peak of the EEG signal, global mean field amplitude, power in various frequency bands, cortico-cortical coherence in various frequency bands, significant current density, phase-locking, and significant current scattering. Measures including spectral power, coherence and connectivity will be used to assess the impact of tACS on EEG dynamics. ANOVA will be used to determine whether these metrics vary as a function of brain region across subjects. The EEG functional connectivity will be assessed using a variety of different

metrics, including cross-correlation coefficient, coherence, synchronization likelihood, transfer entropy, partial directed coherence, and Granger causality. These metrics will be calculated on EEG recorded during the eyes-closed resting state. Values will be obtained for multiple segments of data of various window sizes (generally on the order of 4 to 10 seconds to permit analysis of low-frequency bands) before and after rTMS and tACS; consequently, multiple data segments will be available in each period. Each statistical analysis will be based on a significance threshold equal to p.0.05, correction for multiple comparisons will be applied when needed by using Bonferroni correction, False Discovery Rate (FDR) or network-based statistics (NBS).

PET analysis –

Studies have suggested that the test-retest reliability of amyloid-PET measurement is high, with an intraclass correlation of 0.99, and a relative measurement error of 3% (C. R. Jack et al., 2013). The annual rate of change in the global and regional amyloid ratio varies as a function of patient clinical status and baseline *relative standard uptake values* (SUVR) values normalized to uptake in the cerebellum (Lopresti et al., 2005)), but is generally either nonsignificant or small but positive, typically on the order of 0.05 units/yr, and asymptotes in patients with cognitive deficits and high amyloid levels (C. R. Jack et al., 2013; Clifford R. Jack et al., 2009; Villain et al., 2012; Villemagne et al., 2013), suggesting that major changes are unlikely over the short time period between baseline and post-tACS testing. Furthermore, significant decreases in amyloid (> 0.05 units/yr) are rare, particularly in patients with AD (C. R. Jack et al., 2013), suggesting that decreases above this magnitude would represent a reliable measure of tACS effect. The same trajectory applies to Tau and microglia progression, with differences in the reference region(s) used for normalization of SUVR (e.g. cerebellum and pons for Tau PET data).

Given the exploratory nature of the study, and potential variability in amyloid/TAU localization and levels across patients, analysis will be carried out case by case, by looking at longitudinal changes in PET markers via paired t-tests. Also, since tACS will be delivered on just up-to-two regions showing amyloid deposition (within the same hemisphere or in different hemispheres), a within-patient comparison will be also performed by looking at changes in Amyloid, TAU and Microglia SUVR on the stimulated regions *vs* not stimulated ones.

C. Subject Selection

Subjects will be recruited from the Cognitive Neurology Unit at BIDMC, through clinicaltrials.gov and from local referring practitioners. The inclusion and exclusion criteria include specific exclusions from the PET imaging protocol at MGH. If participants do not meet these criteria, they will not continue in this study and will not be referred to the MGH PET imaging protocol.

Inclusion Criteria:

- Clinical Diagnosis of mild to moderate AD*
    - Mini Mental State Examination (MMSE) ≥ 18
        - Mild AD ≥ 21
        - Moderate AD 18-20
    - Demonstration or history of memory impairments.

* Confirmation of diagnosis will be made by the study MD based on a holistic consideration of the participant's cognitive evaluation and history.

- Amyloid positive PET imaging
- At least 45 years old
- On a stable dose of medications for memory loss including cholinesterase inhibitors (e.g. donepezil, rivastigmine or memantine) as defined as 6 consecutive weeks of treatment at an unchanging dose
- Minimum of completed 8th grade education
- No history of intellectual disability

Exclusion Criteria:

- Current history of poorly controlled migraines including chronic medication for migraine prevention

- Current or past history of any neurological disorder other than dementia, such as epilepsy, stroke (cortical stroke), progressive neurologic disease (e.g. multiple sclerosis) or intracranial brain lesions; and history of previous neurosurgery or head trauma that resulted in residual neurologic impairment.

  Non-cortical disease such as confluence white matter changes (including lacunar infarcts < 1cm) and asymptomatic, subacute, cerebellar infarcts may be included upon review of a medically responsible neurologist.

- Past or current history of major depression, bipolar disorder or psychotic disorders, or any other major psychiatric condition.

- Contraindication for undergoing MRI or receiving TMS or tACS,

- >50 mSv of radiation exposure for research within the past year (PET imaging exclusion)

- History of fainting spells of unknown or undetermined etiology that might constitute seizures.

- History of seizures, diagnosis of epilepsy, history of abnormal (epileptiform) EEG or immediate (1st degree relative) family history of epilepsy; with the exception of a single seizure of benign etiology (e.g. febrile seizure) in the judgment of the investigator.

- Chronic (particularly) uncontrolled medical conditions that may cause a medical emergency in case of a provoked seizure (cardiac malformation, cardiac dysrhythmia, asthma, etc.).

- Metal implants (excluding dental fillings) or devices such as pacemaker, medication pump, nerve stimulator, TENS unit, ventriculo-peritoneal shunt, cochlear implant, unless cleared by the study MD.

- Substance abuse or dependence within the past six months.

- Medications will be reviewed by the responsible MD and a decision about inclusion will be made based on the following: The patient's past medical history, drug dose, history of recent medication changes or duration of treatment, and combination of CNS active drugs.

- All female participants that are pre-menopausal will be required to have a pregnancy test; any participant who is pregnant or breastfeeding will not be enrolled in the study.

- Subjects who, in the investigator's opinion, might not be suitable for the study
- A hair style or head dress that prevents electrode contact with the scalp or would interfere with the stimulation (for example: thick braids, hair weave, afro, wig)

STUDY VISIT SCHEDULE									
Procedures	**Screening/Baseline[A] (Baseline Days 1-8)**		**Daily Visits (Treatment Days 1-20) 4 Weeks, Mon-Fri[D]**				**Follow-up[B] (Follow-Up Days 1-7)**	**1-mon Follow-up**	**3-mon Phone Follow-up**
	1	2-8	Week 1	Week 2	Week 3	Week 4	1-7		
Neurological exam	X*								
Demographic Review	X*								
Medical Hx and Medication Review	X*						X		
TMS/tACS/MRI safety questionnaires	X*								
MMSE	X*						X		
Inclusion/ Exclusion Review	X*								
CDR	X								
ADAS-Cog	X						X		
CGIC	X						X	X	X
Cognitive Testing	X						X	X	X
Edinburgh Handedness	X								
Pregnancy Test	X								
Saliva	X								
fMRI	X						X		
Amyloid PET[C]	X						X		
Tau PET[C]	X						X		
Microglia PET[C]	X						X		
Lumbar Puncture (optional)	X						X		
Blood Draw	X						X		
TMS-EEG	X						X		
tACS-EEG	X						X		
Review of adverse events	X		X	X	X	X	X		
tACS (1 hour)			X	X	X	X			
Cognitive Assessment			X	X	X	X			
Participant Experience Assessment			X	X	X	X	X		
EEG								X	

* = these procedures must be completed on screening day 1
A = screening and baseline procedures may be completed over 6-8 days according to resources, timing and subject tolerability
B = follow-up procedures may be completed over 5-7 days according to resources, timing and subject tolerability
C = will be conducted at MGH
D = It is possible that the study visits will span a 5 week period if there are make-up visits and/or a subject does not begin on a Mon

B4. POSSIBLE BENEFITS

Participants will undergo an amyloid PET scan, which would not be covered by their insurance and may be critical for diagnostic clarity. They will receive the amyloid PET scan results which will be shared with their provider if they desire. A growing number of studies have demonstrated the potential benefit of this knowledge for patient clinical care. Current standard of care recommendations for Alzheimer's Disease include maintaining an active lifestyle and social engagement, therefore the daily sessions, routine and interaction with the study staff may also benefit the patient. It is not possible to predict whether the subjects' cognitive symptoms will improve from participation in this study. Participation in the study may help others in the future as a result of knowledge gained from the research.

B5. POSSIBLE RISKS AND ANALYSIS OF RISK/BENEFIT RATIO

TMS

TMS has been used in a growing number of laboratories worldwide for over twenty years, and safety guidelines have been developed to prevent potential side effects. In the present study, all recommended safety precautions for TMS will be strictly adhered to by the investigators. The subject will be monitored for adverse reactions during and following the treatment.

Even though all known safety precautions will be implemented as part of the present protocol, the following side effects and discomforts might occur:

More Common:

- Up to 20%-40% of subjects undergoing TMS experience headaches or neck pain, which are believed to be due to muscle tension. All prior cases of headaches induced by TMS have promptly resolved with a single dose of acetaminophen (Tylenol®) or aspirin. In some cases TMS may cause facial discomfort on the same side of stimulation.

Rare:

- Subjects may have a seizure caused by TMS. If a seizure occurs, it will occur during the TMS application itself, not after. Repetitive TMS can induce a seizure even in the absence of pre-existing brain lesions, epilepsy, or other seizure risk factors, both in patients and healthy subjects. From the several thousands of studies that have used TMS to date, a total of 16 cases have been reported, of which 9 cases occurred after the 1998 safety guidelines. Based on the available data, the reported risk of seizures is less than 1 in 1000 for repetitive TMS. For the theta-burst rTMS stimulation protocol used in the study, the risks appear to be negligible. There is only one reported instance of a possible seizure triggered by theta-burst rTMS. Further, this event occurred with intensities higher than will be used in the current study. Nevertheless, this is a very concerning complication and to make the subjects' risk as small as possible, the investigators will follow precautions that are recommended by the International Society for Transcranial Stimulation and mentioned in the 2008 updated safety guidelines of The Safety of TMS Consensus Group. Subjects will be carefully observed throughout the study by an investigator who has undergone specific training on how to identify and respond to a seizure. Additionally, a licensed neurologist will be available to evaluate participants if needed.

  However, a seizure could occur. If a seizure occurs, the subject would receive prompt treatment by a neurologist. The laboratory is equipped with all necessary emergency materials that might be required to control a seizure and prevent injury from it. In addition, in the event of a seizure, subjects will receive appropriate medical examination and follow-up at the Cognitive Neurology Unit and the Division of Epilepsy and Clinical Neurophysiology at the Beth Israel Deaconess Medical Center.

- TMS produces a loud clicking sound when a current is passed through the stimulation coil. This loud click can result in ringing in the ear and temporary shifts in the ability to determine the pitch and loudness of sounds, if no protection is used. In order to prevent this possible side effect, participants will wear earplugs or noise cancelling earphones that block the noise of the TMS. Animal and human studies have shown that earplugs can effectively prevent the risk of hearing disturbance due to TMS. The forms of TMS that we will use in this study have never caused hearing problems.

- Syncope can occur due to anxiety and psychophysical discomfort during testing and treatment with TMS. This is reported less than seizure activity but the true number may be higher due to under-reporting. Subjects will be monitored for feeling any signs or symptoms of a pending syncopal event (i.e. feeling dizzy or lightheaded). If these symptoms occur, TMS will immediately be stopped and the subject will be assisted.

- TMS could induce short-term changes in memory, attention and other cognitive and mental functions. Safety studies conducted found these events to be rare and transient.

- Acute psychiatric effects have been described in patients receiving rTMS. Although single cases suggest a causal relationship between rTMS and mania, the overall rate (13 cases) across 53 randomized controlled studies in depression appears to be low (0.84% mania for active rTMS vs. 0.73% for Sham rTMS) and even below natural switch rates in patients with bipolar disorders receiving mood stabilizers (2.3–3.45%). Similarly, cases of rTMS-induced psychotic symptoms, anxiety, agitation, suicidal ideation and insomnia have been reported, but it is unknown whether these occur at higher rates compared to the natural course of disease being treated or associated with other interventions. Psychotic symptoms and suicidal ideation have never been described in normal subjects during or after rTMS. Subjects with psychiatric problems will not be included in this study, so mood changes are not anticipated.

- Dental Pain: The possibility of dental pain during rTMS has been reported. This potential adverse effect of TMS would occur during the application of the stimulation itself. Should such discomfort occur, we encourage the participant to alert the study investigator. The stimulation session will be immediately terminated, and the participant will be encouraged to seek a dental evaluation. This is a very rare occurrence, but it may point to the presence of a cavity that may require care. This adverse effect should not lead to any lasting problems or complications.

- There is no evidence of teratogenic affects at the level of magnetic field that is applied during TMS. Additionally, based upon modeling, the reduction in magnetic field from the head to the abdomen demonstrates that there is not any meaningful exposure of electromagnetic field or risk of induction of any current in a fetus. The electromagnetic field is only engaged by the TMS operator when the stimulation is being applied. Therefore, when the machine is not being activated, the magnetic field is not present. The rare risk of a maternal generalized seizure induced by TMS is of potential harm to a fetus. The overall risk of a generalized seizure induced by TMS is thought to be less than 1:1000 to 1:10,000. Furthermore, a seizure has never been induced by TMS in a pregnant woman and indeed pregnancy reduces the risk of seizures due to hormonal effects on brain cortical excitability. Thus the true risk is quite small. Nevertheless, if a subject is a woman and capable of becoming pregnant, a pregnancy test will be done to verify that she is not pregnant.

- Finally, even though TMS has been used in several laboratories worldwide since 1984, there could be some unexpected complications.

tACS

Noninvasive transcranial current stimulation (tCS) has been safely used in human for decades. It has been used as well safely by the applicants. These noninvasive current stimulation techniques use battery-powered current generator devices that have a built-in circuitry to limit the current above a certain level, typically 2 mA. tCS, in particular transcranial Direct Current Stimulation (tDCS) has been widely used during the last decade demonstrating non-significant risk to participants (Brunoni et al., 2011; Iyer et al., 2005; M. a Nitsche et al., 2008; M. A. Nitsche & Paulus, 2011). In a comprehensive review of studies published from 1998 to 2011 that was authored by an international panel of experts, it was concluded that "Extensive animal and human evidence and theoretical knowledge indicate that the currently used tDCS protocols are safe" (Nitsche et al. 2003;Nitsche and Paulus 2011). This study uses alternating currents (i.e. tACS) which results in less net charge being applied than in tDCS. There is limited reporting of side effects from tCS using alternating currents (tACS) in the literature. Studies that have used tACS, have reported adverse effects that are similar in nature to effects described in the tDCS literature, for example, headache, sensations under the electrodes and visual sensations (Antal et al. 2008;Brignani et al. 2013;Feurra et al. 2011a). Adverse effects that have been described in the tDCS literature are described here in addition to the tACS reports to offer a conservative assessment of possible adverse effects. The most common side effects associated with tCS according to the most recent data available are:

1) Sensations reported by subjects under the electrode:

(These sensations can sometimes continue throughout and for a brief period following completion of the tCS but usually resolve shortly after the initiation of tCS)

- Mild tingling (20-70%)
- Light itching (30-40%)
- Slight burning (10-22%)
- Discomfort or mild pain (10-18%)

2) Effects reported that occur only during tCS:
   - Visual sensation during switching on and off the stimulation (11%),

3) Other effects that can occur both during and after tCS include:
   - Moderate fatigue (35%)
   - Skin redness (30%)
   - Headache (10-15%)
   - Difficulties in concentration (11%)

4) Additionally the following rare side effects have been described:
   - Nausea (3%)
   - Nervousness (<5%)
   - Ringing in the ear (<1%)
   - Changes in the activity of the prefrontal region have the potential to induce acute changes in mood. Hypomania has been reported in a few patients receiving tDCS for bipolar disorder (Loo et al., 2012) and depression (Arul-Anandam et al., 2010) but never in normal controls. Subjects with a history of a psychiatric disorder will be excluded from the study.
   - Transient visual disturbance (2%)
   - Tingling in upper extremities (<1%)
5) Although it has never been reported in tCS, seizures are a theoretical risk.
   There is no evidence that including participants with non-cortical disease such as confluent white matter changes – including lacunar infarcts < 1cm – and asymptomatic, subacute, cerebellar infarcts, would increase seizure risk. tDCS has been studied extensively in research as an intervention in cortical post-stroke patients without any report of seizures. In addition, the current/electrical field induced in the brain during tCS affects the cortex, not the deeper brain structures, which is a known limitation of this method of brain stimulation, thus the development

of surgically placed deep brain stimulators. In a recent two-day consensus meeting of experts from neurophysiology, neurology, cognitive neuroscience and psychiatry, which took place in Göttingen, Germany in 2016, the stimulation related risks and the risk-benefit ratio of tCS was reviewed based upon an extensive review of the literature to date. A resulting consensus paper (Antal, 2017), supports that a tCS related seizure has never been reported in the literature, including studies conducted in older subjects and post-stroke subjects.

In addition, across all methods of noninvasive brain stimulation (TMS, tDCS, tACS, tRNS), there is no evidence of a cumulative dose effect promoting seizure risk (Rossi, 2009, Antal, 2017). The risk of seizure is dependent upon the parameters used in a given session. The stimulation parameters utilized in this study follow published guidelines (Antal, 2017) and the single session parameters are the same in the prior approved protocol (IRB# 2017P000373, PI Dr. Santarnecchi). The only difference regarding the stimulation regimen between this new protocol and the prior approved one relates to the increase of the number of daily sessions from 2 to 4 weeks, and as mentioned there is no evidence of cumulative dose effect promoting seizure risk.

To reduce the incidence of adverse reactions, the stimulation will be ramped up and down at the stop and start of the stimulation, as suggested in current recommendations (Nitsche et al., 2003).
A licensed MD will be available by page during the study visits.

Lumbar Puncture

Lumbar puncture may be associated with pain during the procedure, but this is usually temporary and limited to the lower back. In about 5% of older adults who undergo LP, headache can occur but this is typically mild and will resolve with over-the-counter analgesics. Less commonly (1-4%), a persistent low-pressure headache (with features of headache only on standing) may develop, and the rate is generally lower in older subjects. Potential but rare risks (less than 1%) of lumbar puncture include infection, bleeding into the CSF space, damage to nerves in the back, and death.

To minimize the risk of post-LP headache, a small gauge or atraumatic (Sprotte) needle will be used. We will follow-up with patients one day after the procedure. If a post-LP headache persists, additional treatment, e.g. with fluids and analgesics will be administered. If persistent post-LP headache develops, the patient will be referred for appropriate follow-up clinical care by our study Neurologist.

MRI

MRI is a painless and safe technique that can be used to investigate brain structure and functioning. Participants will be screened for MRI exclusionary criteria. We will follow all the guidelines and recommendations endorsed by the National Advisory Mental Health Council (NAMHC) Workgroup on MRI Research Practices that was convened on September 14, 2005.

There are no known or foreseeable risks or side effects associated with conventional MRI procedures except to those people who have electrically, magnetically or mechanically activated implants (such as cardiac pacemakers) or to those who have clips on blood vessels in their brain. Participants will therefore be screened very carefully to exclude the possibility that they have any such devices and/or implants and will be excluded from participation in the event that they do. There are no known additional risks associated with functional MRI. Both the conventional and the functional MRI systems have been approved by the FDA and will be operated within the standards reviewed and accepted by the FDA.

A magnetic resonance scan might be uncomfortable if participants are a) prone to claustrophobia (fear of enclosed spaces); b) do not like to lie still for a period of time, or c) do not like banging or beeping sounds. The researcher will explain the procedure and if a potential participant expresses any doubt about a), b), or c), he/she will not be included in the study. All participants will be told that they can notify

the researcher in charge of the scan if they feel uncomfortable, and ask to be taken out of the scanner at any stage. Participants will be given earplugs to reduce scanner noise and will be able to contact the investigator at any time during the scan via a squeeze ball and intercom system, and can be taken out of the scanner at any stage of the imaging procedure immediately upon request.

PET

The PET imaging will occur under a separate protocol submitted at MGH, although participation in the protocol is a required part of this study, therefore exposure to radiation is a risk of the study. Participants will be informed of the risk of the PET imaging during the consent for this protocol to allow them and their study partner the opportunity to consider those risks and willingness to participate in this protocol and the protocol at MGH. The total exposure and risk as detailed in the MGH protocol is as follows:

Intravenous Catheter:

An intravenous catheter will be placed to inject contrast agent. The subject may feel some discomfort and have some bruising, swelling or bleeding at the site where the needle goes in. Rarely an infection may occur at this site. If infection does occur, it will be treated.

Radiation Exposure:

The total maximum radiation exposure for this study (6 PET/CT scans) will be as follows:

Radiotracer	Dose		CT Dose	Total pre and post intervention
	mCi	mSv	mSv	Total mSv x2
[^{11}C]PiB	15	2.61	1	7.22
F-18 T807	10	9.18	1	20.36
C-11 PBR28	15	3.66	1	9.32
				36.9 mSv total study dose

If testing results indicate the subject should not have the PBR28 scan, then total amount of radiation exposure for 4 PET scans is as follows:

Radiotracer	Dose		CT Dose	Total pre and post intervention
	mCi	mSv	mSv	Total mSv x2
[^{11}C]PiB	15	2.61	1	7.22
F-18 T807	10	9.18	1	20.36
				27.58 mSv total study dose

If subjects have been exposed to radiation in the past 12 months, they will be asked to inform the investigators or study staff If it is determined that the prior radiation exposure exceeds the current guidelines for research exposure (i.e., 50 mSv/year), the subject will not be enrolled in this study or the MGH PET protocol.

The radiotracers [^{11}C]PiB, [^{18}F]T807, and [^{11}C]PBR28 will be produced by the MGH PET Core Production facility. No adverse reactions have been linked to the use of these radioligands. The safety standards approved by the MGH Radiation Safety Committee for the use of radioligands will be strictly followed. There is an extremely small risk of allergic reaction to the radiotracers used in this study.

During the scan, the patient will be in an enclosed space and this may cause some people to experience claustrophobia. Participants will be able to contact staff using a squeeze ball at any time and can be taken out of the scanner at any stage of the procedure at their request.

EEG

There are no known serious risks associated with EEG, nor does the combination of tACS and EEG appear to confer an increased risk of harm. Subjects may rarely experience scalp irritation from the electrode placement, but this is almost universally transient (lasting only a day or two).

Blood Draw

The risks of a blood draw include pain or soreness at the insertion site and bruising.

Cognitive Testing

Undergoing detailed, comprehensive cognitive testing can cause distress or anxiety in some individuals who may feel they are answering questions incorrectly, stressed by the task demands, tired and fatigued by sustained attentional demands, etc.

Genetic Testing

The only potential risk for genetic testing relates to accidental release of protected health information (PHI). All saliva samples will be immediately labeled using only a unique study identifier. The master code for the samples will be maintained in a password-secured file on the BIDMC network drive. Participants will not be informed of the results of the genotyping, nor will that information be entered into their medical history. See **B8. DATA SECURITY** section for more details.

Data Safety Monitoring Plan

The DSMP consists of a comprehensive plan for monitoring the participant throughout the study. This will be accomplished by daily assessments of cognition and of adverse effects, both expected and unexpected. The study will be monitored both by a Data Safety Monitoring Board (DSMB) and by an independent medical monitor (IMM).

Ongoing Cognitive Assessment:
The application of multiple session tACS stimulation has been less frequently studied as compared to tDCS. Prior tACS studies have not reported negative cognitive effects, including a study applying multi-day stimulation in healthy subjects (Polania, Nitsche, Korman, Batsikadze, & Paulus, 2012; Santarnecchi et al., 2016). Additionally, in our current SHARP protocol (IRB # 2017P000011), we and our collaborating sites have run up to 373 healthy subjects through a multi-day tACS protocol without any noted significant problems. Despite this, we will collect a baseline MoCA evaluation that will be repeated at the end of each stimulation visit. If the score drops 4 points or more from baseline, the covering neurologist will be alerted to assess the participant further, as necessary.

Adverse Event Monitoring
Adverse effects will be collected from the start of the experimental protocol to the end of study participation. All adverse events, regardless of attribution to tACS or pre/post assessments, will be collected and recorded using a standard adverse event form. Participants will be asked, in an open-ended way, about the presence of any such events on a daily basis. Additionally, a standard questionnaire for tACS-related adverse effects will be completed in the period after every tACS session. Intensity of each adverse event will be graded as mild, moderate or severe. If an event occurs that is not expected (e.g. is not described in the research protocol or consent form), that indicates a change from baseline in cognition, and/or requires immediate attention, such as a seizure, the PI (or covering investigator) will be informed in real time to assess the event, advise on immediate care of the participant and to determine the necessary reporting steps. Any events that are serious or

unexpected in nature, severity or frequency as compared to the risks described in the study plan will be reviewed by the principal investigator or designee (e.g. a co-investigator) to determine the relationship of the event to the study. Reportable events will be submitted to the IRB per determined policies and will be reported to the DSMB and IMM as described below.

Adverse Event Communication with MGH for the PET/CT protocol

Adverse events related to the PET/CT imaging protocol at MGH will be monitored and collected by study staff at MGH. Following the completion of each subject, the staff at MGH will share adverse events that were captured by their study team with the BIDMC study team. This information will be included in the summaries provided to the DSMB and IMM. A serious or unexpected adverse event that requires reporting to the MGH IRB per the MGH site PI will be reported to the BIDMC study staff in parallel with the MGH reporting procedures. These events will be shared with the DSMB and IMM in real time as will any local reportable events. If any adverse events are noted by BIDMC study staff that immediately follow or may be related to the PET study visits (e.g. bruising or redness at an IV site), this information will be communicated to the MGH study staff once it is noted either in person, via phone or secure email.

DSMB

A DSMB will be appointed for this study as described in Part P. A report will be provided to the DSMB at least every 6 months for review. The report will include enrollment information, a summary of adverse events, a summary of the ongoing cognitive assessments and any other data that the group determines to be necessary. Serious and unexpected adverse events will be reported to the DSMB simultaneously with reporting to the BIDMC IRB within the designated IRB guidelines. For example, a serious adverse event will be reported by fax or e-mail within 1 business day, followed by a written report within 7 days. The DSMB reports will be shared with the IRB during continuing review or sooner if the DSMB requests action, such as pausing the study or amending the protocol.

IMM

The IMM is an independent physician with relevant experience who will independently monitor the study for risks and safety monitoring in a timely fashion. Expected AE's and cognitive assessment data will be collected by the study team and provided to the IMM following the completion of the first two subjects and will then be provided this information every 6 months unless otherwise requested. The IMM will have access to the study participant's research records and will have access to interview the participant and study team as needed. Serious and unexpected adverse events will be reported to the IMM simultaneously with reporting to the DSMB and BIDMC IRB within the designated IRB guidelines. The IMM will provide a report to the study team following report reviews and assessments. The IMM will discuss any suggested changes to the research protocol with the study team as a result of his/her oversight. Safety and adverse effect data from this study will be shared with the IMM along with any other additional materials as per request. The IMM will have access to PHI. The IMM reports will be shared with the IRB during continuing review or sooner if the DSMB requests action, such as pausing the study or amending the protocol. **Please see the attached "Independent Medical Monitor Plan" for review and approval.**

U. S. Army Medical Research and Materiel Command (USAMRMC) Office of Research Protections (ORP) Human Research Protection Office (HRPO) Reporting

All unanticipated problems involving risk to subjects or others will be promptly reported to the HRPO by telephone (310-619-2165), by email (usarmy.detrik.medcom-usammc.other.hrpo@mail.mil), or by facsimile (301-619-7803). A complete written report will be provided following the initial notification. Suspensions, clinical holds (voluntary or involuntary), or terminations of this research by the Institutional Review Board (IRB), the institution, the Sponsor, the DSMB, the IMM or regulatory agencies will be promptly reported to the USAMRMC ORP HRPO.

General Safety Plan

A licensed physician, credentialed at BIDMC will be available by pager during all tACS and TMS visits at BIDMC. Furthermore, the person applying tACS and TMS will have training in basic life support (BLS) with the availability of emergency equipment. We will monitor patients in detail during and after delivery of tACS, and TMS using an approach drawn directly from suggested guidelines.

Withdrawal Criteria

Subjects may be withdrawn from the trial if:

- A serious adverse event occurs.
- The investigator considers it, for safety reasons, in the best interest of the subject that he/she be withdrawn.

B6. RECRUITMENT AND CONSENT PROCEDURES

Recruitment

Participants will be recruited through the Cognitive Neurology Unit at BIDMC, clinicaltrials.gov and through local neurologists and memory clinics, such as the Massachusetts Alzheimer's Disease Research Center, Beth Israel Deaconess Medical Center Needham, and the MGH Memory and Disorders Unit and using the Alzheimer's Organization's Trial Match system (alz.org/trialmatch). Local neurologists and in-network physicians will be contacted with a letter that will be mailed or e-mailed informing them of the study including a copy of the flyer that they can distribute to interested patients and their families. Patients will also be recruited using ICD-9 and ICD-10 codes for Alzheimer's via Clinical Query 2 using the CQ2 Data Download Tool to identify potential participants. Medical records will be reviewed of the identified patients to assess for study eligibility. Recruitment letters will be used to provide interested participants and providers information about the study. Flyers and information sheets will be posted in the community, or distributed to community members (e.g. senior centers, local libraries) and providers. A brochure will be distributed to provide more information to potential participants and providers. Once a potential subject is identified that fits the inclusion/exclusion criteria, the physician on record at BIDMC will be contacted to ask for their permission to contact the subject by phone, email, or with a letter containing details of the study on behalf of the physician on record. Medical records will be reviewed for identified participants during the screening process to assess for study eligibility. Medical records for external patients will be obtained from their providers to confirm mild to moderate AD diagnosis (including any amyloid PET imaging results).

A basic template of language will be used in a brochure in the Cognitive Neurology Unit, and may be used for other recruitment purposes, such as sharing it with outside organizations to place in newsletters or on websites (i.e. local senior centers, community centers, Alzheimer's Associations, and/or Alzheimer's support groups). The brochure for the Cognitive Neurology Unit (CNU) contains information for memory loss studies in the CNU that can easily be updated to reflect studies actively recruiting, adding new, approved studies and removing those closed to enrollment. Each study will have language approved for this brochure under each individual protocol as is described here.

If a participant has had a positive PET scan for amyloid in the past it may be obtained for use in the study.

Consent

At the screening visit, a co-investigator will review the consent form in its entirety with prospective subjects and allow the participant and study partner ample time to review the consent. The PI, or other designated study MD, will complete the review of the consent with the participant and study partner and will answer any questions regarding the study. The purpose of the study, the procedures used, the requirements of participation, risks and benefits, the right to withdraw at any time will be reviewed and any questions will be answered. A family member/significant other (study partner) will be involved in

the informed consent process. The patient will be told that at any point, he/she may choose to terminate the study for any reason and that he/she has the option not to participate in the study. Any and all questions will be answered to the best of the researcher's capabilities. If any answers are unknown, this will be stated to the potential subject. The informed consent will be signed by the subject or the subject's legally authorized representative if appropriate. Signed consent forms will be placed in the participant's research file and a copy will be given to the participant.

Subject Protection

As we will be recruiting patients with AD, they may be considered vulnerable due to their cognitive status. As a neurologist will be reviewing the pre-screening materials and meeting with the subject and obtaining consent, they will have insight into the subject's ability to grasp the consent. There is no evidence that the cognitive deficits of mild dementia, as defined by an MMSE of 20 or greater, interfere with a participant's ability to comprehend study procedures and any risks that they may entail (Buckles et al., 2003). This information is likely to be known prior to consent from pre-screening and clinic notes. According to the same research, a patient's ability to fully comprehend all procedures does decrease with moderate dementia (MMSE 18-20), All attempts will be made to assure that the participant understands the study. In all cases, a study partner will be required for participation in this study. The study partner will be present for the consent so that they can assist the subject with decisions about study participation during the consent and throughout the study based upon the subject's wishes. All participants and their partner will be provided with a visual study schematic as an additional method of communicating the study plan. If a subject is not capable of providing consent, then the legally authorized representative will provide consent. Prospective subjects will be excluded if they express clear objections to participating or if the study neurologist has concerns about their ability to understand the procedures of the study.

Study Partner Consent

All participants will have someone who agrees to be a study partner. A study partner consent script will be reviewed by a member of the study staff with the designated study partner. This script will provide details about the role of study partner and will allow for the study partner to ask questions about the study and about their role throughout their loved ones participation. If the study partner stops participation partway through the study, the participant will choose a new study partner and the new partner will be consented.

B7. STUDY LOCATION

Privacy

Subjects will be seen in a private lab space in the Berenson Allen Center or the Clinical Research Center for consent, all screening activities, cognitive testing, baseline assessments and tACS and TMS visits assuring privacy during all of the evaluations.

Physical Setting

Recruitment and screening procedures will take place by study staff at the Beth Israel Deaconess Medical Center in the Berenson-Allen Center for Non-Invasive Brain Stimulation or the Clinical Research Center. Data will be stored at BIDMC and data analysis will occur at BIDMC.

B8. DATA SECURITY

To safeguard confidentiality and the privacy of protected health information, each study subject will be assigned a unique code number. A separate log linking the patient's name with study number and identifiers will be kept in a password-protected data file, accessible only by the study investigators. Names will not be provided to external sources other than the staff on the MGH PET protocol once the subjects has signed consent and agreed to be in the study. No will any identifying information will be published in which a participant could be distinguished.

PET scan images will be sent to BIDMC using a secure FTP site. Any paper forms from MGH (e.g. adverse event forms) will be scanned and sent to BIDMC via encrypted email. All paper records regarding this research project will be stored in the locked offices of the research study team, located within the BA-CNBS at BIDMC. Medical records from outside providers will be requested and obtained via secure email or fax. Data from this study and from the MGH PET study will be entered into and stored in the REDCap electronic data management system or stored in a data file on the BIDMC server. An extensible open-source imaging informatics software platform (XNAT) will be used to store subjects' de-identified MRIs and PET images. The XNAT will be hosted and managed by BIDMC and will be kept behind the firewall for security purposes.

Information that will be shared with MGH for the PET study will include PHI that is required to register the participant, pre-screening for inclusion (e.g. past radiation exposure), medical history and medications. The MRI that is obtained for this study will be shared with MGH staff as well to assist with analysis of the PET imaging data. All information will be provided via secured email and/or secure file transfer.

Blood samples that are collected for DNA screening for the microglial PET will be sent to the Boston Children's Hospital IDDRC genetic core lab for processing. The samples will be de-identified with the key remaining on the BIDMC server behind the firewall and accessible by study staff only.

Saliva samples that are collected for DNA will be sent outside of BIDMC for analysis. Samples are assigned a random number identifier. The samples are individually labeled with this identifier along with the collection date. A key that links the saliva sample to the subject is maintained on a password protected data sheet in a folder in the BIDMC server. One sample will be sent to an outside lab for analysis, and the other sample will be maintained at BIDMC in case the other sample is unable to be analyzed, or for future research. When samples are sent out for analysis, a manifest is sent with the samples that includes the de-identified randomly assigned number only. The results are reported back to the study team using this number. The study team re-identifies the results after receiving the de-identified report. The outside lab has no way of identifying the subjects and does not retain or store any of the samples. Once the saliva is analyzed, the sample is destroyed by the outside lab.

B9 Multi-Site Studies

Is the BIDMC the coordinating site? ☒ Yes ☐ No

Is the BIDMC PI the lead investigator of the multi-site study? ☒ Yes ☐ No

Research teams from both locations will communicate via phone, email or conference calls on a monthly basis or more, as needed. In these interactions, teams will assess safety and review study progress. Study staff from BDMC will accompany the subject to all study visits at MGH allowing for ongoing communication between sites.

Adverse events that occur at MGH will be communicated to BIDMC study staff for completion of DSMB, IMM, HRPO and IRB communication as described above in the "Risks and Analysis" section

under the "Data Safety Monitoring Plan". Additionally, any adverse events noted in follow-up to PET procedures (e.g. bruising noted at IV insertion site) by the BIDMC staff will be communicated to MGH staff as described above.

All data analysis will be managed by BIDMC although PET imaging analysis will be completed in a collaborative manner between the sites. Regular meetings will be scheduled as described above with ad hoc meetings scheduled as needed. BIDMC will manage protocol amendments that affect the overall study aims. Recommended changes that affect the PET imaging protocol will be communicated to the MGH study team either in the monthly meeting or via phone and/or email. Any amendment needs that are identified by the MGH team for the PET imaging protocol will be communicated to the BIDMC team in the same manner.

B10 Dissemination of Research Results

The results of the study will be primarily disseminated through peer-reviewed journals and scientific conferences.
If a participant is interested in being contacted directly, we will send a follow-up e-mail thanking the subject for their participation in the study. In this follow-up e-mail, we will inform subjects that results of the study will be available on our website tmslab.org as all publications are available on the website. Subjects will also be informed that they can "like" us on Facebook as we regularly post information about new publications, presentations and publicity on the site.

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