Feasibility and Safety of a High-Frequency Transcranial Alternating Current Stimulation Intervention for Amyloid-β Reduction in Alzheimer's Disease

NCT03290326

05/15/2018

PART B
STUDY DESCRIPTION

TITLE OF PROTOCOL	**Feasibility and Safety of a High-Frequency Transcranial Alternating Current Stimulation Intervention for Amyloid-β Reduction in Alzheimer's Disease**
Principal Investigator	**Emiliano Santarnecchi, PhD**

B1. PURPOSE OF PROTOCOL

Alzheimer's Disease (AD) 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.

The proposed study aims to show that transcranial alternating current stimulation (tACS) is feasible and can be performed in patients with mild AD, and will assess for an initial signal in terms of decreased amyloid, improved neurophysiological markers, and improvement in cognition. In subsequent studies, we will show that this reduction of amyloid is sustained, prevents disease progression, and improves long-term cognitive outcomes. This study will thus provide the critical first step in the development of a novel intervention to prevent and treat Alzheimer's Disease.

Our central hypothesis is that 10 daily sessions of 40 Hz tACS is safe in patients with mild AD, will significantly decrease cerebral amyloid levels on amyloid PET imaging, and this decrease in amyloid will be correlated with improvement on electrophysiological measures of brain function, and on cognitive testing.

Specific Aim #1: To demonstrate that 10 sessions of 40 Hz tACS stimulation is safe and reliable in patients with mild AD.
We hypothesize that tACS will be well tolerated by all subjects, without any significant side effects.

Specific Aim #2: To demonstrate that 10 sessions of 40 Hz tACS stimulation decreases amyloid levels on PET imaging in patients with mild AD.
We hypothesize that 40 Hz tACS will result in a significant decrease in amyloid burden in the stimulated area.

Specific Aim #3: To demonstrate that 10 sessions of 40 Hz tACS stimulation improve resting-state EEG background activity, EEG connectivity, and performance on cognitive testing.
We hypothesize that 40 Hz tACS will result in a normalization of brain activity with increased high frequency (gamma) EEG activity and increased brain network connectivity, 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.

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); that tau is isolated to the lateral temporal lobes without amyloid, and spreads outside the lateral temporal lobes in patients with amyloid; and that neurodegeneration and clinical symptoms are 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.

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). The same authors have also determined that in presymptomatic AD mice, induction of gamma activity remarkably prevents subsequent neurodegeneration and behavioral deficits, suggesting that gamma induction may represent a novel and powerful therapeutic approach for AD. This opens to the possibility of modulating gamma activity in humans, potentially leading to the same beneficial effects observed in mouse models. 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).

That being said, drug-based interventions do not allow for precise targeting of Aβ 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 only affect visual and auditory cortices. PET imaging allows for characterization of individual patterns of Aβ deposition (see Figure 2), 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 preliminary data on an individualized intervention to reduce Aβ with greater spatial accuracy, by means of transcranial alternating current stimulation (tACS).

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) (Figure 3). 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.

B3. DESCRIPTION OF RESEARCH PROTOCOL

A. Study Design – Overview, Methods, Procedures

Overview

This is a safety and feasibility study of tACS in patients diagnosed with early AD. Individuals who have had amyloid PET imaging and have evidence of cerebral amyloid burden will be recruited. Individuals with a diagnosis of mild Alzheimer's Disease and suspected amyloid burden will also be recruited and undergo a PET scan during the screening process. All subjects will receive 10 sessions of active tACS targeted on a case-by-case basis to the regions of 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 Clinical Research Center. We aim to study 10 individuals with AD with evidence of increased cerebral amyloid burden on amyloid PET imaging. Up to 20 participants will be enrolled to account for attrition. Each subject's participation in the study will consist of up to 18 visits: 4-5 days for screening/baseline procedures as described below (these baseline assessments may happen over 4-5 days to accommodate scheduling and the participant), 10 tACS study visits, and 4-5 days for follow-up assessments. Subjects will undergo baseline cognitive assessment, structural and functional MRI characterization, resting-state EEG measurement and amyloid PET scan (if not previously obtained). 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 10 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 10 sessions, subjects will then repeat the baseline assessments, followed by repeat amyloid PET imaging to assess for changes in amyloid burden.

Procedures

Screening and Baseline Visit (Visits 1-4)

During the screening and baseline visit, participants will provide informed consent and complete the following procedures:

- Neurological exam*
- Demographic review*
- Review of medical, psychiatric and medication history, including review of diagnosis*
- tACS and TMS safety questionnaires*
- MMSE*
- The Clinical Dementia Rating (CDR) scale to assess severity of dementia*
- Inclusion/Exclusion criteria review*
- MRI screening questionnaire
- Alzheimer's Disease Assessment Scale Cognitive Subscale (ADAS-Cog)
- Baseline Clinical Global Impression of Change (CGIC)

- 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)
- 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 number span will be done in all cases. The MoCA 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
- Modified Edinburgh Questionnaire to determine handedness
- Pregnancy test for females of child-bearing potential
- 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 visit #2 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
- PET Imaging
- Lumbar puncture** (optional)
- Blood draw**
- TMS-EEG plasticity assessment
- tACS-EEG recording with cognitive tasks

The screening and baseline visits will occur over 4-5 days. All of the screening activities necessary for determining inclusion and safety will be completed on the first day as indicated by the asterisks above (*). The remainder of the activities will be planned according to scheduling, resources, timing and subject tolerability. It is possible that there would be an additional visit due to these issues. It is possible that a baseline activity will happen on the first day of stimulation prior to the tACS (e.g. a questionnaire).
**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 such as 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. 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 (visits 5-14)

Study visits 5-14 will be conducted in the Berenson-Allen Center for Noninvasive Brain Stimulation at BIDMC. Participants will undergo 10 days (weekdays) of tACS. Participants will be allowed to miss one visit for a total of 9 days of tACS. If they miss more than one visit, additional sessions will be added on if it is within a reasonable timeframe as determined by the investigator, in order to reach a total of 9 sessions. 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.
- 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…..).

Follow Up Visit (Visits 15-18)

The following will occur at the follow up visit within approximately 1 week after the completion of the tACS study visits:

- Cognitive Evaluation that includes the MMSE, NACC-UDS questionnaires and cognitive assessments completed at baseline, RAVLT, ADAS-Cog, and CGIC.
- Repeat baseline UDS questionnaires
- PET Imaging – A follow-up PET scan will be obtained to assess the change in amyloid burden in the stimulated target region
- fMRI
- Lumbar puncture (optional)
- Blood draw
- TMS-EEG plasticity assessment
- tACS-EEG with cognitive tasks
- Adverse event review and follow-up
- Review of medications and changes in medical history
- At the completion of the study, subjects will be queried about their overall experience with the study using the Participant Experience Assessment

It is possible that the follow-up visit will be conducted over 4-5 days due to availability of the PET scanner and resource availability.

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

Telephone Follow-up After (1 month and 3 months after completion of visit 18)
The following will occur via telephone call at two time points, approximately 1 month and 3 months after the participant completes the in-person follow-up visits. for assessment of any longer term changes:

Measures that were collected during prior visits will be completed via telephone:

- 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)

For study participants who already completed the study we plan to complete the telephone follow-up retroactively. If the participant has already passed the 1 month follow-up mark, but not the 3 month mark we plan to call them at two time marks-- immediately and at the 3 month mark. If the participant has already passed both the 1 month and 3 month marks, we plan to complete the follow-up assessment with them at one time mark, following up with them as soon as possible.

Methods

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 member 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 point 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 th eMoCA with the removal of the pencil/paper and visual portions of the assessment. The test takes approximately 10 minute 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 50 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, participants whose abdomen, shoulder, or hip circumference is greater than 180cm may need to be excluded from the study.

PET

An amyloid PET scan will be conducted at baseline. A follow-up PET scan will be obtained after the completion of the tACS sessions. PET imaging with Florbetapir-[18]F injection will be performed on BIDMC's Siemens Biograph m64 multidimensional helical PET-CT Scanner. A 10 minute emission scan, acquired with a 128 x 128 matrix (zoom 2) in 1 x 10min frames, will be obtained 30-50 minutes after intravenous injection of 10 mCi (370 Mbq) of Florbetapir F18 (or Florbetaben).

The follow-up PET imaging to be done in this study is the equivalent of a clinical PET study using a ligand that is FDA approved for assessing Aβ burden. This ligand is the same as the ligand used in the PET obtained prior to enrollment. The scan will be read by a nuclear medicine physician and a report will be prepared.

Participants will be informed of their baseline amyloid imaging results as the scan must be positive to be included in the study. The study investigator will inform the participant if the scan was positive or negative and will be clear with the participant that this scan was collected for a research study. If participants would like to understand how their results relate to their diagnosis or care, they can opt to have the results shared with their provider. The scans are all collected and read in the same manner that they would be if done clinically. If participants are in agreement that they would like to share the scan results with their provider, the study team will work with them to provide this information to their care provider. If they do not have a provider who is comfortable with relating the results to the subject's care or if they do not have a provider, the study team will work with them to arrange an appointment in the Cognitive Neurology Unit.

If patients have had a clinical or research amyloid PET at BIDMC, we may use these results as a measure of amyloid progression.. If patients have had a clinical PET at an outside hospital, we may ask them to obtain a copy to compare with the current study.

TMS – EEG: Brain plasticity and responsiveness to stimulation

The TMS-EEG measures will be collected at baseline and then again following the 10 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 (10 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.

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 and batch shipped to an outside laboratory for analysis.

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 to an outside laboratory 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 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 10 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 to the regions of tracer uptake on the amyloid PET scan on a case-by-case basis. 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.

Stimulation will be slowly ramped up/down at the beginning/end of each stimulation session to minimize skin sensation. tACS can elicit phosphenes - a sensation of light caused by excitation of the retina by mechanical or electrical means rather than by light. 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. We anticipate that a low percent of participants will experience this sensation (10%).

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 amyloid according to the aforementioned SUVR threshold. In order to get valuable pilot data about changes in PET markers, we aim to recruit up to 20 participants to account for attrition with 10 expected to enroll in the study. This would allow for a final sample of 5/6 fully characterized participants who will complete the tACS

intervention as well as pre-post multimodal evaluation. As for the quantification of changes in amyloid load, we will compare individual *relative standard uptake values* (SUVR) before and after tACS, according to established threshold values. PET Amyloid levels across brain regions will be quantified using standard uptake values normalized to uptake in the cerebellum (SUVR; (Lopresti et al., 2005)). 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). Subjects will be defined as amyloid positive if the global (across all regions) cortical to cerebellar SUV ratio is greater than or equal to 1.6 (Clifford R. Jack et al., 2008). The annual rate of change in the global and regional amyloid ratio varies as a function of patient clinical status and baseline SUVR, 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-intervention testing. Furthermore, significant decreases in amyloid (> 0.05 units/yr) are rare, particularly in patients with MCI/AD (C. R. Jack et al., 2013), suggesting that decreases above this magnitude would represent a reliable measure of tACS effect. Changes in SUVR will be evaluated by looking at individual trajectories of amyloid change (i.e. decrease in amyloid load or slowing of amyloid build-up) exceeding the threshold.

Data Analysis:
Changes in EEG metrics (e.g. gamma spectral power), amyloid beta levels, 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).

C. Subject Selection

Subjects will be recruited from BIDMC and outside providers and will have had an amyloid positive PET scan, or suspected amyloid burden.

Inclusion Criteria:

- Clinical Diagnosis of mild AD*
    - Clinical Dementia Rating (CDR) = 0.5 - 1
    - Mini Mental State Examination (MMSE) ≥ 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.

- Previous amyloid positive PET imaging or suspected to be amyloid positive
- At least 45 years old
- Minimum of completed 8th grade education
- 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
- 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 confluent white matter changes (including lacunar infarcts < 1 cm) 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,
- 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 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[A] (Day 1-4)**			**Daily Visits (Day 5-14)**										**Follow-up[B] (Day 15-18)**			**Telephone Follow-up (1 mo. and 3 mo. post study)**
	1	2	3	1	2	3	4	5	6	7	8	9	10	1	2	3	
Neurological exam	X*																
Demographic Review	X*																
Medical History Review	X*														X		
Medication Review	X*														X		
TMS & tACS safety questionnaires	X*																
MMSE	X*														X		
CDR	X*																
Inclusion/ Exclusion Review	X*																
MRI Screening Questionnaire	X																
ADAS-Cog	X														X		
CGIC	X														X		X
NACC-UDS	X														X		X – ADL only
WTAR	X*														X		
RAVLT	X														X		
Edinburgh Handedness	X																
Pregnancy Test (if applicable)	X																
Saliva (optional)	X																
fMRI	X														X		
PET	X														X		
Resting State EEG	X																
Lumbar Puncture (optional)	X														X		
Blood Draw	X														X		
TMS-EEG	X														X		
tACS-EEG	X														X		
Review of side effects/ adverse events				X	X	X	X	X	X	X	X	X	X		X		
Cap set up				X	X	X	X	X	X	X	X	X	X				
tACS (1 hour)				X	X	X	X	X	X	X	X	X	X				
Cognitive Assessment				X	X	X	X	X	X	X	X	X	X				X
Participant Experience Assessment															X		

* = these procedures must be completed on screening day 1

A = screening procedures may be completed over 4-5 days according to resources, timing and subject tolerability with the exception of the procedures necessary for determining inclusion and safety as indicated by *

B = follow-up procedures may be completed over 4-5 days according to resources, timing and subject tolerability. A 1 month and 3 month phone follow up of cognition and function will be completed

B4. POSSIBLE BENEFITS

It is not possible to predict whether subjects will benefit directly 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%)
5) Although it has never been reported in tCS, seizures are a theoretical 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, small gauge or atraumatic Sprotte needles 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

This research study involves exposure to radiation from 3 amyloid PET/CT brain scans. Using the standard way of describing radiation exposure, from participating in this study, participants will receive a total of 24mSv. For comparison, the average person in the United States receives a radiation exposure of 0.003 Sv (or 3 mSv) per year from natural background sources, such as from the sun, outer space, and from radioactive materials that are found naturally in the earth's air and soil. The dose that participants will receive from this research study is about the same amount they would normally receive in 8 years from these natural sources. The injection of the radiotracer may cause pain at the injection site and rarely may cause allergic reactions. A crash cart is on hand in the unlikely event the participant has an allergic reaction to the tracer and a nuclear medicine specialist will review the participant's history prior to the procedure.

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. PET imaging is an extremely safe procedure that is used worldwide for clinical diagnostic purposes.

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).

Cognitive Testing

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

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

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

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. This cognitive testing 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.

All subjects will be monitored throughout the study for any adverse reactions in relation to the tACS using the Adverse Reaction Form for tACS. Adverse reactions will be reviewed daily prior to tACS and then following tACS. A DSMB will be appointed for this study as described in Part P. Adverse effects will be collected at BIDMC and will be reported to the DSMB every 6 months for review. 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.

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 standard adverse event forms. Participants will be asked, in an open-ended way, about the presence of any such events. Also, a standard questionnaire for tACS-related adverse effects will be performed in the period after every tACS session. Intensity of each adverse event will be graded as mild, moderate or severe. Events will be medically evaluated as appropriate, including testing and referral.

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

We aim to recruit 10 participants for this study. Participants will be recruited through the Cognitive Neurology Unit at BIDMC, as well as through Clinicaltrials.gov. Participants will also be recruited through the Berenson-Allen Center databases. Individuals who have agreed to be contacted for future studies will be contacted via email or phone call to see if they are interested in participating in the study. Individuals outside of BIDMC with a mild Alzheimer's diagnoses will also be recruited. 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. 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 AD diagnosis (including any amyloid PET imaging results).

A basic template of language will be used in a brochure for 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 website (i.e. local senior centers, community centers, the 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, the PI or a designated co-investigator will review the consent form in its entirety with prospective subjects. The investigator will explain the purpose of the study, the procedures used, the requirements of participation, risks and benefits, the right to withdraw at any time, and answer any questions. A family member/significant other 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 memory problems/early AD, they may be considered vulnerable due to their cognitive status. Although participants have a diagnosis of cognitive impairment, the impairment is mild as determined by an MMSE score of 20 or greater. There is no evidence that the cognitive deficits of mild dementia interfere with a participant's ability to comprehend study procedures and any risks that they may entail (Buckles et al., 2003). As an additional protection, we aim to include the participant's family member/significant other in the consent process. It is not anticipated that the participant's memory loss will progress over the course of the study as the study consists of 16 visits within a relatively short timeframe.

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, nor will any identifying marker be published in which a participant could be distinguished. Data will be entered into and stored in the REDCap electronic data management system or stored in a data file on the BIDMC server.

Medical records from outside providers will be requested and obtained via secure email or fax. 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.

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

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.

References

Amidzic, O., Riehle, H. J., Fehr, T., Wienbruch, C., & Elbert, T. (2001). Pattern of focal gamma-bursts in chess players. Nature, 412(0028–0836 (Print)), 603.

Antal, A., Boros, K., Poreisz, C., Chaieb, L., Terney, D., and Paulus, W. (2008). Comparatively weak after-effects of transcranial alternating current stimulation (tACS) on cortical excitability in humans. Brain Stimulation 1(2), 97-105.

Bath, K. G., & Lee, F. S. (2006). Variant BDNF (Val66Met) impact on brain structure and function. Cognitive, Affective & Behavioral Neuroscience, 6(1), 79-85.

Babiloni, C., Lizio, R., Marzano, N., Capotosto, P., Soricelli, A., Triggiani, A. I., … Del, P. C. (2015). Brain neural synchronization and functional coupling in Alzheimer's disease as revealed by resting state EEG rhythms. Int.J. Psychophysiol., 1872–7697.

Brignani, D., Ruzzoli, M., Mauri, P., and Miniussi, C. (2013). Is transcranial alternating current stimulation effective in modulating brain oscillations? PLoS. One. 8, e56589.

Brunoni, A. R., Amadera, J., Berbel, B., Volz, M. S., Rizzerio, B. G., & Fregni, F. (2011). A systematic review on reporting and assessment of adverse effects associated with transcranial direct current stimulation. The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum (CINP), 14(8), 1133–1145.

Buckles, V.D. et al. Understanding of informed consent by demented individuals. Neurology. 61, 1662-1666 (2003)

Dincheva, I., Glatt, C. E., & Lee, F. S. (2012). Impact of the BDNF Val66Met polymorphism on cognition: Implications for behavioral genetics. Neuroscientist, 18(5), 439-451.

Feurra, M., Bianco, G., Santarnecchi, E., Rossi, A., Rossi, S. (2011). Frequency-dependent tuning of the human motor system induced by transcranial oscillatory potentials. J.Neurosci., 31, 12165–12170.

Feurra, M., Pasqualetti, P., Bianco, G., Santarnecchi, E., Rossi, A., & Rossi, S. (2013). State-dependent effects of transcranial oscillatory currents on the motor system: what you think matters. J.Neurosci., 33(1529–2401(Electronic)), 17483–17489.

Feurra, M., Paulus, W., Walsh, V., & Kanai, R. (2011). Frequency specific modulation of human somatosensory cortex. Front Psychol., 2(1664–1078 (Electronic)), 13.

Fertonani, A., Ferrari, C., and Miniussi, C. (2015). What do you feel if I apply transcranial electric stimulation? Safety, sensations and secondary induced effects. Clin. Neurophysiol. *126*, 2181–2188.

Frohlich, F., & McCormick, D. A. (2010). Endogenous electric fields may guide neocortical network activity. Neuron, 67(1097–4199), 129–143.

Hebert, L. E., Weuve, J., Scherr, P. A., & Evans, D. A. (2013). Alzheimer disease in the United States (2010-2050) estimated using the 2010 census. Neurology, 80(19), 1778–1783.

Herrmann, C. S., Frund, I., & Lenz, D. (2010). Human gamma-band activity: a review on cognitive and behavioral correlates and network models. Neurosci.Biobehav.Rev., 34(1873–7528, 981–992.

Iaccarino, H. F., Singer, A. C., Martorell, A. J., Rudenko, A., Gao, F., … Tsai, L.-H. (2016). Gamma frequency entrainment attenuates amyloid load and modifies microglia. Nature, 540(7632), 230–235.

Iyer, M. B., Mattu, U., Grafman, J., Lomarev, M., Sato, S., and Wasserman, E. M. (2005). Safety and cognitive effect of frontal DC brain polarization in healthy individuals. Neurology 64(5), 872-875.

Jack, C. R., Jr., Knopman, D. S., Jagust, W. J., Shaw, L. M., Aisen, P. S., Weiner, M. W., … Trojanowski, J. Q. (2010). Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. Lancet Neurol., 9(1474–4465 (Electronic)), 119–128.

Jack, C. R., & Holtzman, D. M. (2013). Biomarker Modeling of Alzheimer's Disease. Neuron, 80(6), 1347– 1358.

James, B. D., Leurgans, S. E., Hebert, L. E., Scherr, P. A., Yaffe, K., & Bennett, D. A. (2014). Contribution of Alzheimer disease to mortality in the United States. Neurology, 82(12), 1045–1050.

Kanai, R., Chaieb, L., Antal, A., Walsh, V., & Paulus, W. (2008). Frequency-dependent electrical stimulation of the visual cortex. Curr.Biol., 18(1879–0445 (Electronic)), 1839–1843.

Kasten, F. H., Dowsett, J., & Herrmann, C. S. (2016). Sustained Aftereffect of α-tACS Lasts Up to 70 min after stimulation. Frontiers in Human Neuroscience, 10.

Musaeus, C. S., Shafi, M. M., Santarnecchi, E., Herman, S. T., & Press, D. Z. (2017). Levetiracetam Alters Oscillatory Connectivity in Alzheimer's Disease. Journal of Alzheimer's Disease, 1–12.

Nitsche, M. A., Liebetanz, D., Lang, N., Antal, A., Tergau, F., and Paulus, W. (2003). Safety criteria for transcranial direct current stimulation (tDCS) in humans. Clinical Neurophysiology. 114(11), 2220-2222.

Nitsche, M. A., Cohen, L. G., Wassermann, E. M., Priori, A., Lang, N., Antal, A., and Pascual-Leone, A. (2008). Transcranial direct current stimulation: State of the art 2008. Brain Stimulation, 1(3), 206– 223.

Nitsche,M.A., and Paulus,W. (2011). Transcranial direct current stimulation--update 2011. Restorative Neurology and Neuroscience, 29(6), 463-492.

Ozen, S., Sirota, A., Belluscio, M. A., Anastassiou, C. A., Stark, E., Koch, C., & Buzsaki, G. (2010). Transcranial electric stimulation entrains cortical neuronal populations in rats. J.Neurosci., 30(1529–2401 (Electronic)), 11476–11485.

Peña-Gomez, C. *et al.* APOE status modulates the changes in network connectivity induced by brain stimulation in non-demented elders. *PloS One* **7,** e51833 (2012).

Polania, R., Nitsche, M. A., Korman, C., Batsikadze, G. & Paulus, W. (2012). The Importance of Timing in Segregated Theta Phase-Coupling for Cognitive Performance. Current Biology, 22, 1314–1318.

Pontecorvo, M. J., Devous, M. D., Navitsky, M., Lu, M., Salloway, S., Schaerf, F. W., … for the 18F-AV-1451-A05 investigators. (2017). Relationships between flortaucipir PET tau binding and amyloid burden, clinical diagnosis, age and cognition. Brain, aww334.

Risacher, S. L. *et al.* The role of apolipoprotein E (APOE) genotype in early mild cognitive impairment (EMCI). *Front. Aging Neurosci.* **5,** 11 (2013).

Ruffini, G., Fox, M. D., Ripolles, O., Miranda, P. C., & Pascual-Leone, A. (2014). Optimization of multifocal transcranial current stimulation for weighted cortical pattern targeting from realistic modeling of electric fields. Neuroimage., 89(1095–9572 (Electronic)), 216–225.

Santarnecchi, E., Biasella, A., Tatti, E., Rossi, A., Prattichizzo, D., & Rossi, S. (2017). High-gamma oscillations in the motor cortex during visuo-motor coordination: A tACS interferential study. Br. Res. Bull, 131, 47–54.

Santarnecchi, E., Brem, A. K., Levenbaum, E., Thompson, T., Kadosh, R. C., & Pascual-Leone, A. (2015). Enhancing cognition using transcranial electrical stimulation. Current Opinion in Behavioural Sciences, (1879–0445 (Electronic)), 171–178.

Santarnecchi, E., Muller, T., Rossi, S., Sarkar, A., Polizzotto, N. R., Rossi, A., & Kadosh, R. C. (2016). Individual differences and specificity of prefrontal gamma frequency-tACS on fluid intelligence capabilities. Cortex, 75(1879–0445 (Electronic)), 33–43.

Santarnecchi, E., & Rossi, S. (2016). Advances in the Neuroscience of Intelligence: from Brain Connectivity to Brain Perturbation. Span. J Psychol, 19(1988–2904 (Electronic)), E94.

Santarnecchi, Polizzotto, N. R., Godone, M., Giovannelli, F., Feurra, M., Matzen, L., … Rossi, S. (2013). Frequency-Dependent Enhancement of Fluid Intelligence Induced by Transcranial Oscillatory Potentials. Current Biology, 23(15), 1449–1453.

Schmidt, S. L., Iyengar, A. K., Foulser, A. A., Boyle, M. R., & Frohlich, F. (2014). Endogenous cortical oscillations constrain neuromodulation by weak electric fields. Brain Stimul., 7(1935–861X 878–889.

Tatti, E., Rossi, S., Innocenti, I., Rossi, A., & Santarnecchi, E. (2016). Non-invasive brain stimulation of the aging brain: State of the art and future perspectives. Ageing Res. Rev, 29(1872–9649, 66–89.

Verret, L., Mann, E. O., Hang, G. B., Barth, A. M., Cobos, I., Ho, K., … Palop, J. J. (2012). Inhibitory interneuron deficit links altered network activity and cognitive dysfunction in Alzheimer model. Cell, 149(1097–4172 (Electronic)), 708–721.

Brunoni, A. R., Amadera, J., Berbel, B., Volz, M. S., Rizzerio, B. G., & Fregni, F. (2011). A systematic review on reporting and assessment of adverse effects associated with transcranial direct current stimulation. *The International Journal of Neuropsychopharmacology / Official Scientific Journal of the Collegium Internationale Neuropsychopharmacologicum (CINP)*, *14*(8), 1133–45. https://doi.org/10.1017/S1461145710001690

Freitas, C., Farzan, F., & Pascual-Leone, A. (2013). Assessing brain plasticity across the lifespan with transcranial magnetic stimulation: why, how, and what is the ultimate goal? *Front Neurosci.*, *7*(1662–4548 (Print)), 42. https://doi.org/10.3389/fnins.2013.00042

Iyer, M. B., Mattu, U., Grafman, J., Lomarev, M., Sato, S., & Wassermann, E. M. (2005). Safety and cognitive effect of frontal DC brain polarization in healthy individuals. *Neurology*, *64*(5), 872–5. https://doi.org/10.1212/01.WNL.0000152986.07469.E9

Jack, C. R., Lowe, V. J., Senjem, M. L., Weigand, S. D., Kemp, B. J., Shiung, M. M., … Petersen, R. C. (2008). 11C PiB and structural MRI provide complementary information in imaging of Alzheimer's disease and amnestic mild cognitive impairment. *Brain*, *131*(3), 665–680. https://doi.org/10.1093/brain/awm336

Jack, C. R., Lowe, V. J., Weigand, S. D., Wiste, H. J., Senjem, M. L., Knopman, D. S., … Petersen, R. C. (2009). Serial PIB and MRI in normal, mild cognitive impairment and Alzheimer's disease: implications for sequence of pathological events in Alzheimer's disease. *Brain*, *132*(5), 1355–1365. https://doi.org/10.1093/brain/awp062

Jack, C. R., Wiste, H. J., Lesnick, T. G., Weigand, S. D., Knopman, D. S., Vemuri, P., … Petersen, R. C. (2013). Brain -amyloid load approaches a plateau. *Neurology*, *80*(10), 890–896. https://doi.org/10.1212/WNL.0b013e3182840bbe

Lopresti, B. J., Klunk, W. E., Mathis, C. A., Hoge, J. A., Ziolko, S. K., Lu, X., … Price, J. C. (2005). Simplified Quantification of Pittsburgh Compound B Amyloid Imaging PET Studies: A Comparative Analysis. *Journal of Nuclear Medicine*, *46*(12), 1959–1972.

Nitsche, M. a, Cohen, L. G., Wassermann, E. M., Priori, A., Lang, N., Antal, A., … Pascual-Leone, A. (2008). Transcranial direct current stimulation: State of the art 2008. *Brain Stimulation*, *1*(3), 206–23. https://doi.org/10.1016/j.brs.2008.06.004

Nitsche, M. A., & Paulus, W. (2011). Transcranial direct current stimulation--update 2011. *Restor Neurol Neurosci.*, *29*(6), 463–92.

Polania, R., Nitsche, M. A., Korman, C., Batsikadze, G., & Paulus, W. (2012). The importance of timing in segregated theta phase-coupling for cognitive performance. *Curr.Biol.*, *22*(1879–0445 (Electronic)), 1314–1318. https://doi.org/10.1016/j.cub.2012.05.021

Santarnecchi, Muller, T., Rossi, S., Sarkar, A., Polizzotto, N. R., Rossi, A., & Cohen Kadosh, R. (2016). Individual differences and specificity of prefrontal gamma frequency-tACS on fluid intelligence capabilities. *Cortex*, *75*, 33–43. https://doi.org/10.1016/j.cortex.2015.11.003

Villain, N., Chételat, G., Grassiot, B., Bourgeat, P., Jones, G., Ellis, K. A., … Villemagne, V. L. (2012). Regional dynamics of amyloid-β deposition in healthy elderly, mild cognitive impairment and Alzheimer's disease: a voxelwise PiB–PET longitudinal study. *Brain*, *135*(7), 2126–2139. https://doi.org/10.1093/brain/aws125

Villemagne, V. L., Burnham, S., Bourgeat, P., Brown, B., Ellis, K. A., Salvado, O., … Masters, C. L. (2013). Amyloid β deposition, neurodegeneration, and cognitive decline in sporadic Alzheimer's disease: a prospective cohort study. *The Lancet Neurology*, *12*(4), 357–367. https://doi.org/10.1016/S1474-4422(13)70044-9