**Effectiveness of a Non-Invasive, Low-Intensity Brain Stimulation Approach in Addressing Emotional Regulation & Memory**

**(CogT BEEM Study)**

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## 1. PURPOSE OF STUDY

Neuropsychiatric symptoms (NPS) are a distinctive set of behavioral disturbances that are prevalent in mild cognitive impairment (MCI) and Alzheimer's disease (AD), worsening of which, particularly, can accelerate patients' cognitive and functional decline and cause significant distress to caregivers.[1,2] NPS often co-exist, rendering the need to understand the shared biological mechanisms underlying multiple NPS, which is crucial for the development of effective interventions to address them simultaneously. Notably, we recently discovered a neural circuit shared by MCI and AD patients with multiple NPS; the circuit is composed of 10 frontal-temporal-striatal regions[3] and is highly related to both study partner-rated Neuropsychiatric Inventory Questionnaire (NPI-Q) and AD pathology (indexed by cerebrospinal fluid Aβ/ptau ratio).[3] The **goal of this proof-of-concept mechanistic intervention study** is to confirm the causal relationship between this neural circuit and various NPS, by modifying a key region within the neural circuit [i.e. left precentral gyrus (LPG), critical for regulating visual attention[4]] with anodal transcranial direct current stimulation (tDCS).

Why focus on the LPG via tDCS to understand the contribution of this shared neural circuit to NPS? Our previous work on NPS-shared neural circuit shows that while different NPS are associated with the same 10 frontal-temporal-striatal regions, the strength of functional connectivity (FC), as well as its directionality, varies for different NPS. For example, the strength of most FCs in MCI/AD patients with a depressive symptom is lower than that in patients without this symptom, while the strength of most FCs are higher in patients with an anxiety symptom. Across NPS, however, LPG is the only region that has lower FCs with frontal and striatal regions within the circuit whenever a NPS is present.[3] LPG, parallel to C3 in the 10/20 EEG system,[5] is one of the most frequently examined and effective tDCS targets modifying affective, psychiatric, and psychosomatic symptoms, in addition to motor function.[6-8] Administering anodal tDCS also enhances the strength of FCs linking LPG to frontal and limbic regions that are part of the NPS-shared neural circuit.[9-12] Notably, our feasibility study demonstrated our readiness and safety of administrating anodal tDCS to C3 in older adults. Synthesizing these separate lines of evidence, we **hypothesize** that administering anodal tDCS to C3 in patients with NPS will result in enhanced activation of LPG and reorganization of NPS-shared neural circuit, both of which will improve NPS.

**Aim 1. Determine the effect of tDCS on NPS-shared neural circuit**. **H:** After intervention, the active group, relative to the sham group, will have a greater increase in LPG activity, indexed by greater activation in LPG in response to a visual attention task (H1a) and greater increase in resting FC strength between LPG and other regions within the NPS-shared neural circuit (H1b).

**Aim 2. Determine the relationship between NPS-shared neural circuit and study partner-report NPS**. **H:** After intervention, the active group, relative to the sham group, will have greater improvement in study partner-rated NPS in terms of lower severity and fewer co-existing symptoms (H2a). Changes in LPG task-induced activity combined with an LPG-seeded NPS-shared neural circuit will contribute to the

improvement in study partner-rated NPS across groups (H2b).

**Exploratory aim. Examine the relationship between NPS and the coherence between structural and functional aspects of the NPS-shared neural circuit**. Cumulative literature suggests that white matter (WM) tracts play a critical role in supporting and shaping brain function.[13] It is often assumed that FC is dependent on underlying anatomical connections,[13-15] with decreases in WM integrity in dementia related to reduced functional efficiency.[16] The magnitude of correlation between the structural and functional aspects of NPS-shared neural circuit may implicate the severity of NPS. Here, we will collect diffusion tensor imaging (DTI) at baseline, as part of MRI data acquisition, and examine whether the effects of tDCS on NPS-shared neural circuit and study partner-rated NPS depend on WM integrity. Exploring this question will provide broaden our mechanistic understanding of NPS.

We propose that probing the LPG via tDCS provides a way to experimentally test the causal relationship between our previously discovered NPS-shared neural circuit and study partner-rated NPS. The proposed research is highly innovative, while scientifically grounded, for targeting one brain region that may affect multiple NPS. Validating the hypotheses has the potential to support a future R01 study that directly conducts a Stage 2 trial addressing NPS in MCI or AD, thus ultimately improving patients' quality of life and reducing the caregiver burden.

**Exploratory aim 2: Examine blood biomarkers at baseline for predicting cognitive and brain MRI biomarker responses to intervention.** Blood neuropathological (amyloid beta [Aβ] 42, total tau [t-tau], and neurofilament light [NfL]) and neurotrophic biomarkers (brain-derived neurotrophic factor [BDNF], insulin growth factor-1 [IGF-1], and short-chain acylcarnitines [SCACs]) relate to cerebral amyloidosis, neurodegeneration[17-19] and neurogenesis.[20,21] Blood Aβ42 may indicate cerebral amyloidosis,[19] and blood t-tau, and NfL levels have been shown to reflect neurodegeneration across populations (older adults with AD, MCI, or normal cognition).[17,18] Their blood levels also correlate with their cerebrospinal fluid (CSF) levels, as well as several neuroimaging biomarkers (amyloid plaques, AD-related brain atrophy, and brain hypometabolism).[17-19] Decreased blood Aβ42 and increased t-tau and NfL levels were also found in individuals with MCI or AD and were associated with cognitive decline and incident AD over time.[17-19] Preliminary data from colleagues at the University of Minnesota demonstrated that increased baseline plasma t-tau levels correlated with greater cognitive decline in older adults with MCI over time.[22] Moreover, plasma phosphorylated tau (p-tau) is a promising biomarker of neuronal injury. A recent study showed that plasma p-tau levels were better biomarkers for both acute and chronic traumatic brain injury than plasma t-tau levels alone - further providing evidence in support of blood biomarkers as surrogate endpoints for treatment responses in neurological conditions such as aMCI and AD.[23]

Blood neurotrophic factors have been shown to cross the blood-brain barrier to increase hippocampal volume and memory.[20,25-32] Higher plasma BDNF levels were linked to better memory, larger hippocampal volume, and reduced risk for AD.[25,28,29] Whereas higher plasma IGF-1 levels were cross-sectionally associated with better cognition[33] and lower odds of AD,[34] longitudinal data have been inconsistent. However, a recent study showed that higher plasma levels of IGF binding protein-3 (IGFBP-3), which are essential for IGF-1 physiological action,[35] were associated with decreased risk for dementia.[30]

## 2. BACKGROUND AND RATIONALE

Co-existing NPS in MCI, especially those worsening over time, are associated with more rapid cognitive and functional decline and a greater risk of AD.[1] The majority of the existing literature has focused on the neural mechanism and management of individual NPS in MCI/AD.[36,37] Optimal NPS management,

meaning effective management of multiple NPS simultaneously, is a major challenge in MCI/AD care.[2]

**Feasibility of administrating tDCS in older adults:** Previously, 14 cognitively normal older adults (65-85 years) were randomly assigned to active vs. sham tDCS group. TDCS was applied for 20 minutes (as described in the protocol below) while subjects performed, in random order, two computer-based visual attention tasks that also recruit working memory (1-back) and inhibition (color test in Stroop). Participants were asked to make responses as accurately and quickly as possible (10 minutes per task, **Figure 1A**). Active tDCS was anodal stimulation of C3 with cathode on Fp2 (**Figure 1B**). There were no adverse effects. The two groups were similar in baseline global cognition, age, education, and sex. The 20-minute tasks were segmented into four 5-minute blocks with intra-individual variability in reaction time (IIVRT) computed within each block, ensuring the comparability of cognitive performance between the two types of tasks. IIVRT was calculated using standard deviation divided by averaged reaction time of all the trials with correct responses. The active condition induced significantly better cognitive performance (indexed by lower IIVRT) for the last three blocks, compared to the sham condition (**Figure 1C**).

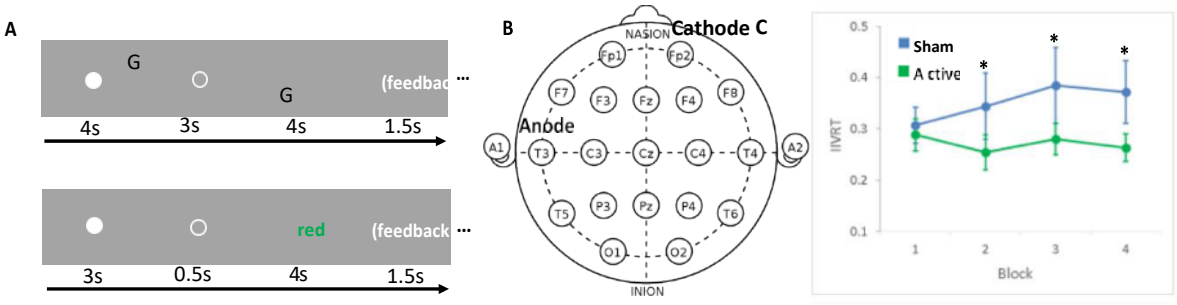


**Figure 1. tDCS pilot study. A.** Visual attention tasks (upper: 1-back; lower: Color test in Stroop); **B.** location of the tDCS electrodes; **C.** cognitive performance across four 5-minute blocks between sham and active groups. * p < .05.

We propose to examine the influence of LPG, a region within the NPS-shared neural circuit and a region known to regulate visual attention, on the NPS-shared neural circuit and study partner-rated NPS. In the proposed study, we capitalize on a non-invasive brain stimulation process to manipulate the activation and FC strength seeded in LPG. This design will provide an understanding beyond the correlational relationship between the neural circuit and NPS in patients with MCI. TDCS non-invasively induces a low direct current in the cortical region of interest (ROI), with a potential neural mechanism of inducing synaptic changes that affect neural network activity, reflected in changes in both activation and strength of FC, as well as changes in visual attention-dominant tasks.[38] LPG is one of the primary tDCS targets for obtaining effects on psychosomatic and affective symptoms[6-8]; furthermore, tDCS can easily target (i.e., on the cortical surface) and modulate (i.e., produces large effects) the region. Applying an average of 20 minutes of continuous tDCS can yield acute effects for up to 90 minutes, while 2-4 weeks of sessions can yield effects for up to 6 months.[39-42] Most importantly, tDCS is safe and non-invasive, thus, these characteristics together make tDCS an ideal manipulation technique to modify LPG.

**Scientific premise and hypothesis:** This proposal aims to determine the causal relationship between our recently discovered NPS-shared neural circuit and study partner-rated NPS by stimulating a region within this circuit (LPG). We integrate the evidence from existing literature and our preliminary work that are potentially relevant to what is proposed here: (1) a worsening pattern of NPS in MCI is most

disturbing for cognitive and functional decline[1]; (2) a neural circuit (i.e., NPS-shared neural circuit) supports multiple NPS and links to study partner-rated NPS and AD pathophysiology[3]; (3) the strength of FCs within the NPS-shared neural circuit varies across individual NPS, with LPG exhibiting greater FC strengths with frontal and striatal regions within the circuit but weaker FC strengths with other regions, the latter of which is linked to higher chances of presenting NPS[3]; (4) LPG is a hub region that regulates visual attention and processing speed[4,43,44]; (5) anodal tDCS targeting LPG enhances brain synchronization and whole-brain resting-state functional connectivity between LPG and its neighboring regions (particularly frontoparietal regions) or between LPG and caudate[9-12]; (6) anodal tDCS targeting LPG also modifies affective, psychiatric, and psychosomatic symptoms (e.g., humor, appreciation, anxiety, pain)[6-8].

We hypothesize that anodal tDCS stimulating LPG can affect LPG activation (indexed by enhanced neural efficiency during the visual attention task) and its connectivity with other regions within the NPS-shared neural circuit, which, in turn, will reorganize the NPS-shared neural circuit and improve study partner-rated NPS (**Figure 2**). Validating these hypotheses will extend separate bodies of literature on the symptomatic understanding of multiple NPS, neural mechanisms underlying NPS, and the impact of tDCS on neural and behavioral effects, as well as facilitate an explicit elaboration of a shared neural mechanism underlying multiple NPS in MCI. This elaboration has strong potential to inform treatment development for managing multiple NPS simultaneously by addressing the shared neural circuit as the primary therapeutic target. This proposal's significance is further underscored by the implications of our model for potentially preventing AD pathology: our pilot work suggests the NPS-shared neural circuit predicts the trajectory of AD pathology progression, indexed by a change in cerebrospinal fluid Aβ/ptau ratio. If the neural circuit is modifiable via tDCS applied to the key region within the circuit, this approach may be applicable to delaying AD pathology from developing and benefit other clinical domains, such as cognitive deficits and impaired functional independence, thereby potentially slowing overall AD progression.

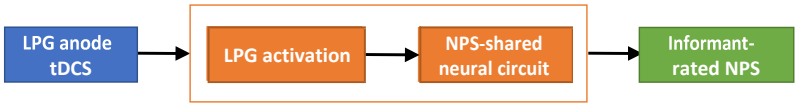


**Figure 2. Conceptual framework**

## 3. ADMINISTRATIVE ORGANIZATION

Study-related appointments will be conducted on-site at University of Rochester Medical Center locations. Subjects will be given the option to do the tDCS interventions at the subject's home or at our lab. During home visits, study team members will assess the environment (ex. dog barking, TV on, etc.) and adjust accordingly depending on the specific situation. Regardless of intervention location, a trained study team member will be administering all tDCS interventions. All in-person assessments for this research study will be conducted in Dr. Lin's CogT Lab (located at the Annex Building). All MRI appointments will be conducted at the Rochester Center for Brain Imaging/Center for Advanced Brain Imaging and Neurophysiology (RCBI/CABIN). Baseline blood collection will be conducted at URMC's Clinical Research Center (CRC).

## 4. STUDY DESIGN

This is a Stage 0 study for mechanistic understanding. We will recruit older adults with study partner-rated NPS that has worsened in the past 2 years. Our previous study shows that this will capture

the most detrimental type of NPS.[1] We will focus on the MCI group, since understanding and modifying NPS among this group may assist in slowing the progression of dementia. See Table 1 for the overview of assessment and intervention timeline.

- Double-blind randomized controlled trial of 40 participants and their study partner subjects; all eligible and interested intervention subjects will be enrolled and undergo a baseline assessment, MRI scan, and blood draw; PI, staff conducting follow-up assessments and intervention subjects and their study partner subjects will be blinded. Interventionists and data managers will know randomization.
- After baseline, all intervention subjects will be randomly assigned to one of two groups - active anodal or sham C3 tDCS. A MATLAB block-based randomization code will be used to randomly assign study ID to group. Each study ID-group pairing will be printed and placed inside a sealed envelope labeled with only the study ID to ensure blinding. After an intervention subject and their partner subject finish baseline assessments, the interventionist or data manager will open the randomization envelope to assign the respective study ID.
- All intervention subjects will undergo a total of fourteen (14) 20-minute tDCS sessions over the course of four weeks: ideally, five times a week for two weeks, followed by twice a week for an additional two weeks. However, we will extend the intervention for additional two weeks for make-up sessions.
- Assessments will occur at baseline, within 2-week after finishing intervention (post-intervention assessment), and 4 to 6 weeks after post-intervention assessment (follow-up assessment).
- Unblinding will occur upon the completion of the follow-up assessment. We will not provide those in the sham group with the active tDCS procedure.

Primary endpoints:

- Resting-state and visual attention task-related fMRI; [45]

fMRI data will be collected using a gradient echo-planar imaging sequence. Participants will undergo a 5-minute resting-state scan at first, being instructed to relax with their eyes open, followed by a 5-minute event-design 'target among distractors' visual search task. For the task, participants fixate on a black screen for a constant inter-stimulus interval of 1000ms, follow by 5500ms presentation of the search pattern. Participants' task is to search for target symbol " 申 " (which was present for 50% of trials), shown among 6 distractors (e.g., "由", "甲", etc.). Participants are instructed to press one of two response buttons to indicate whether the target was present or absent.

Secondary endpoints:

- Study partner-rated NPS (frequency and severity scores from NPI and NPI-Q[46]).

Other measures:

- Cognitive functions will be measured using tests from three different versions of the computer-based battery Executive Abilities: Measures and Instruments for Neurobehavioral Evaluation and Research (EXAMINER);
- Paper-based measures of cognitive functioning will be measured using two versions of the Rey Auditory Verbal Learning Test (RAVLT, Lists A&B and C&D) and the Brief Visuospatial Memory

Test-Revised (BVMT-R, Forms 1-3);

- Functioning in daily life will be measured with the self-report version of the Activities of Daily Living-Prevention Instrument (ADL-PI-self).
- Fatigue across 5 domains (general, physical, mental, and reduced motivation & reduced activity) will be measured using the Multidimensional Fatigue Inventory (MFI);
- Sleep habits and quality of sleep will be measured using the total score from the Pittsburgh Sleep Quality Index (PSQI);
- Depression will be measured using both the 15- and 30-item versions of the Geriatric Depression Scale (GDS);
- Risk of suicide (current & past) and current suicidal ideation will be measured using Columbia Suicide Severity Rating Scale (C-SSRS);
- Anxiety will be measured using both the state and trait anxiety scales from the State-Trait Anxiety Inventory (STAI);
- Perception of stress will be measured using Perceived Stress Scale (PSS);
- Apathy will be measured using the 18-item self-report and informant-rated Apathy Evaluation Scales (AES);
- Quality of life (QoL) across multiple life domains (including physical health, mood, relationships, activities, ability to perform tasks) will be measured using the 13-item self-report and informant-rated Quality of Life in Alzheimer's Disease scales (QOLAD);
- Cognitive reserve will be measured using Cognitive Reserve Assessment Scale in Health (CRASH);
- Decision making and risk-taking behavior will be measured with the 30-item Domain-Specific Risk-Taking scale (DOSPERT)
- Blood biomarkers collected at baseline will be analyzed and compared to changes of cognitive and MRI brain scan cortical thickness outcomes. Diet history will also be measured by asking three questions assessing the intervention subject's consumption of fish oil prior to the blood draw.
- Medication changes will be assessed by asking the intervention subject for an updated list of their prescribed medications. Of note, although we will ensure the stability of the medication (e.g., anti-depressants, antipsychotics, and/or anxiolytics, memory medication) as part of the eligibility criteria (see section 5.a), we cannot ensure the changes of medications throughout the study procedure, which may interfere with the understanding of the proposed mechanism between brain regions and NPS, therefore, we will consider them confounding factors in later analyses.

Alternate forms of cognitive measures (EXAMINER, RAVLT, BVMTR) will be used across time points (see **Table 1** for details) to avoid practice-effects.

### 4.1. SUBJECT POPULATION

a) **Number of Subjects**: The total sample will include 40 intervention subjects with MCI

(NIA-AA diagnostic criteria[47]) and their study partner subjects (who live with or regularly visit the intervention subjects, one partner subject per intervention subject). We expect to consent and screen approximately 200 intervention subjects with MCI, resulting in a likely screen failure rate of 160 intervention subjects.

b) **Gender and Age of Subjects**: Intervention subjects with MCI must be 60-89 years, with approximately an equal number of men and women enrolled. Study partner subjects must be 18 years or older. **Racial and Ethnic Origin**: We will proactively recruit minority subjects – focusing primarily on African American and/or Hispanic/Latino groups, as both are major minority groups affected by dementia & make up most of the minority population in the Rochester area. We plan to enroll approximately 15% African American intervention subjects (about 6 intervention subjects) and about 5% of intervention subjects who are ethnically Hispanic/Latino (about 2 intervention subjects). Resulting in a total minority make-up of approximately 20% (8 intervention subjects) in our research sample. General demographics (DOB, sex, education) of the partner subjects will function as control variables considered for NPI.

Study partner-rated NPI-Q will be used to identify intervention subjects with NPS that has worsened in the past 2 years: (1) in the past month, presence of $\geq$ 2 symptoms; and (2) compared to 2 years ago, having $\geq$ 1 pre-exist symptom of which severity rating gets worsened, or having $\geq$1 new symptom. We are interested in MCI patients with worsened NPI-Q since they are the groups with highest risk for cognitive and functional decline.[1]

**4.2 STUDY INTERVENTIONS**

Our study is registered at ClinicalTrials.gov (registration # NCT04099524).

**We follow tDCS device related information according to this panel review: [48]**

tDCS will be administered using a 1x1 Low-Intensity Transcranial Electrical Stimulator (tES) device (Model 2001) manufactured by Soterix Medical.

- This device is limited, by federal law, to investigational usage and does not meet any of the 7 exemption categories listed in 21 CFR §812.2. No notification from the FDA to the study sponsor requiring application approval was sent. Therefore, this device is considered to have an approved application as an IDE but will require our Institutional Review Board to make a determination regarding whether the IDE is a significant risk or not. We believe the following information may be relevant in the IRB's risk determination:
    - Meets criteria for investigation of device without significant risk: device is not intended as an implant, does not support or sustain human life, is not used for diagnostic purposes, and does not present any potential for serious risk to the health, welfare, or safety of subjects. Please see Section 9 for further information regarding the reported risks of tDCS usage (all of which are minimal).
    - Device will be labelled in accordance with 21 CFR §812.5 – including manufacturer details, quantity of contents, a cautionary statement on device contents regarding its limitations to investigational use (as required by federal law), and bears no misleading statements
    - Records will be maintained in accordance with 21 CFR §812.140 regarding maintenance

of records, including correspondences, device receipts and usage records, records of each intervention subject's case history (including informed consent, exposure to device), reports of adverse device effects, approved protocol with documented protocol deviations.

- Reports will be made in accordance with 21 CFR §812.150 – including documenting unanticipated adverse device effects and reporting to IRB & sponsor within 10 business days; reporting of withdrawal of IRB approval within 5 business days; progress reports will be submitted at least annually, reports of any deviations from investigational plans, informed consent, final study report, as well as any other information requested by either the IRB or sponsor.
- Study sponsor, investigator, and all other study team members comply with the prohibitions outlined in 21 CFR §812.7 prohibiting promotion and other practices.

**4.2.1 Device Storage & Control & Accounting**: The 1x1 tES device will be stored in a locked cabinet, with all of its original contents and appropriate labelling (as in accordance with 21 CFR §812.5). Only the investigator and project coordinator will have access to this locked device. Along with the device and its components, records for when the device was received by the manufacturer, when and on whom the device was used, who administered the device to the intervention subject, as well as up-to-date staff training and procedural logs will all be maintained by the investigator and project coordinator. Furthermore, all safety precautions regarding storage and usage of device outlined in the manufacturer's manual will be followed and records for compliance with storage, handling, and maintenance will be documented.

We will provide training of the stimulation to our experienced project coordinators prior to their administration of the device on intervention subjects. The training will be provided by co-I Dr. Tadin who is an expert in tDCS and Mia Anthony (an experienced tDCS coordinator working on our feasibility study). Evaluation will be conducted by investigator team before a project coordinator can independently work on it.

**4.2.2 Intervention Procedures**:

For all 40 intervention subjects, tDCS (LPG/C3-anode, orbitofrontal cortex/Fp2-cathode) or a sham intervention will be administered for 4 weeks (1 session per weekday for 2 weeks, and then 2 sessions per week for 2 weeks, for a total of 14 sessions).

If any sessions are missed, make-up sessions will be provided, and the length of intervention period will be extended to ensure everyone receives a total of 14 sessions. Intervention subjects can choose to have the tDCS sessions where they feel comfortable (e.g., CogT Lab or their home). The maximum window for make-up sessions is two weeks.

All intervention subjects will receive anodal tDCS stimulation or a sham intervention for 20 minutes per session, on C3 and the cathode electrode on Fp2 using 10/20 EEG system (**Figure C3B**). tDCS will be applied with a pair of 35 $cm^2$ single-use sponges soaked in approximately 4mL of saline solution on each side (~8mL per sponge) connected to the stimulator. During the 20-minute tDCS session, we will use online tDCS design (i.e., an intervention subject will simultaneously work on the visual attention-oriented task.

**Active tDCS Condition**: We will apply the stimulation for 20 minutes using current at 1.5mA with a ramp up and ramp down period of 30 seconds at the start and end of the session (Of note, the two 30 seconds are counted in the 20-minute session).

**Sham tDCS Condition**: tDCS will ramp up for 30 seconds with 1 mA current and then ramp off within 10 seconds. As 30 seconds is too short for tDCS to have any effects, this will be the sham condition. tDCS is on for 30-40 seconds because that is usually the only time individuals would experience tingling and itching – a factor we aim to equate between experimental and control conditions.

## 5. INCLUSION AND EXCLUSION CRITERIA

### a) Inclusion Criteria:

1. Forty intervention subjects with MCI and comorbid NPS, which have worsened inthe past 2 years (as rated by their study-partner subject's responses to theNPI-Q):

(1) In the past month, presence of $\geq$ 2 symptoms; and

(2) Compared to two years ago, having $\geq$ 1 pre-exist symptom whose severity rating has worsened, or having $\geq$ 1 new symptom;

2. Consensus diagnosis of "mild cognitive impairment due to Alzheimer's disease" basedon 2011 NIA-AA diagnostic criteria by the investigators based on screeninginformation:
   i. Memory deficits at screening: 1 standard deviation (SD) below age- and/or education- corrected population norms for the Rey Auditory Verbal Learning Test (RAVLT, Lists C&D);
   ii. Global memory deficits at screening: Montreal Cognitive Assessment (MoCA, Version 2) total score within the range $18 \leq x \leq 26$, after educational adjustment;
   iii. Preserved activity of daily living: ADL-PI-self total score $\leq 30$;
   iv. Absence of dementia.
3. Stable (same dosage, frequency, type) on memory medications for $\geq$ 3 months before screening;
4. Stable (same dosage, frequency, type) on any anti-depressants, antipsychotics,and/or anxiolytics for $\geq$ 7 days;
5. Community-dwelling: Intervention subjects live in homes or independent- and assisted-living facilities (i.e. – not nursing home residents, due to the large cognitive variabilityin nursing home residents);
6. Aged 60-89 years at screening;
7. English-speaking;
8. Adequate visual and hearing acuity for testing;
9. Verified tDCS and MRI safety: Intervention subject should not have any contraindicationsto either and pass safety screening questions for both (see exclusion section for more information);
10. Capacity to consent, based on responses and ratings to the UCSD Brief Assessmentof Capacity to Consent (UBACC) form for this study;

11. Availability of a study partner subject who spends at least several hours per week with the intervention subject, supervises his/her care, and who is willing to accompany the intervention subject to some study visits and participate in the study;
12. Informed consent for study participation obtained by both the intervention subject and his/her study partner subject;
13. Agree to donate 20mL of blood at baseline, after fasting for at least 8 hours (only water and prescribed medicines).

**Study partner subject inclusion criteria:**

1. Age 18 years or older;

2. English speaking;

3. Has regular contact with MCI intervention subject (at minimum weekly) and be able to answer questions about MCI intervention subjects' current or past well-being ormood.

**b) Exclusion Criteria**:

Intervention subjects may be excluded from enrollment, or have their enrollment deferred until they are eligible, for the reasons listed below. Final decisions regarding enrollment will be determined by the PI on a case-by-case basis.

1. Presence of any neurological or vascular disorders (e.g. – Multiple Sclerosis [MS], Traumatic Brain Injury [TBI], chronic heart failure [CHF], Parkinson's disease[PD]);
2. Clinical diagnosis of dementia as defined by the most recent version of the DSM;
3. Current enrollment in another study aimed at improving cognitive abilities and/or emotional well-being;
4. MRI contraindications (e.g. – pacemaker, implantable cardioverter defibrillator[ICD], aneurysm clips, severe claustrophobia);
5. tDCS contraindications (e.g. – scalp or skin condition, history of migraines, seizures or epilepsy, and/or strokes, TBI), metallic implants, history of adverse effects to previous tDCS or other brain stimulation techniques).

## 6. RECRUITMENT METHODS

We will recruit participants using three main recruitment strategies: 1) clinic referrals, 2) established community partners, 3) communities at large. If those strategies fail to reach our recruitment goals, we may initiate additional strategies after IRB approval.

- **Clinic referrals**: participants will be mainly recruited from local clinics, such as the Memory Care Program (MCP) and UR AD-Care, Research and Education Program (AD-CARE) where Dr. Anton Porsteinsson (Co-I) practices as a geriatric psychiatrist. Other local clinics include the internal medicine and geriatric medicine departments of the University of Rochester Medical Center.
- **Community partners**: We will work with our established community partners e.g. Lifespan, Oasis and YMCA who have helped recruit participants in our preliminary studies. Our community partners will distribute flyers and help arrange talks. **Communities at large**: We will reach out to the broad metropolitan communities by distributing recruitment materials to various venues such as senior centers, churches, libraries, and stores. We will present materials atlocal

conferences sponsored by organizations such as our local Alzheimer's Association chapter, Area Agency on Aging, and Volunteers of America

- **Recruitment of minority groups**: To emphasize the efforts for minority recruitment, we will reach out to Recruitment Consultation, a core service of Clinical and Translational Science Institute (CTSI) for consultation & recruitment strategy planning.
- **CTSI participant registry**: This study will identify potential subjects for recruiting using the UR CTSI Research Participant Registry, STUDY00001978. Subjects will be sent an email including a flyer for the study, and be asked to respond if interested. If subjects respond with interest, that person will be added to our database and screened normally via phone screening.
- **Emergency Department Research Associate Program (EDRA):** Potential subjects for recruiting will also be identified using the EDRA program. This program utilizes undergraduate research enrollers to screen interested subjects coming through the Strong Memorial Emergency Department. Lab staff will train EDRA staff to administer initial screening materials to gain a list of eligible and interested participants. These participants will then be added to our database and contacted by lab staff for further screening measures. All newly hired EDRAs complete CITI module training on Human Subjects Protection, GCPs, and HIPAA in addition to study specific training.

## 7. CONSENT PROCESS

Consent is an on-going process that starts when an intervention subject is first informed about the study and ends when the intervention subject's study participation is completed.

**Verbal Consent for Phone and In-Person screening:** When potential participants contact us, the study staff will first explain the study and obtain their verbal consent to proceed with phone screening, using the phone screening script. A waiver of documentation of consent has been requested for this step because it is conducted over-the-phone so participants will not be able to sign this consent themselves. Staff will also obtain verbal consent from the participants' study partner subjects before proceeding with their part of the phone screening. During phone screening, the opportunity to complete secondary screening and assessments over the phone will be offered.

**EDRA Recruiters:** Participants who are recruited through the EDRA program will have their initial phone screening materials and verbal consents completed by the ED recruiters. ED recruiters will undergo training by lab staff in order to administer this measures. Participants recruited this way will also sign a permission to contact form to allow lab staff to contact for additional study measures and enrollment procedures.

**Consent and Re-consent:** The initial signed paper consent will be obtained during the in-person interview by the intervention subject. (Screening Step 2). The partner subject will be consented either at an in-person appointment or via the mail if secondary screening appointment is completed over the phone. Secondary screening over the phone is included to ensure partner subject safety. The intervention subject and study partner subject may complete their secondary screening appointment and consented on separate dates. If the intervention subject completes their in-person screening appointment first and is deemed ineligible, the study partner subject will not proceed with their secondary screening and will not be consented to the study.

1) Explanation of the study: During the second screening interview, the

staff will explain the study in detail to the participants and their study partner subject, including the study purpose, procedures, time commitment, randomization, data collection, risks, benefits, privacy, confidentiality, compensation, voluntary nature, and contact persons for questions and concerns. Emphasis will be placed on explaining the risks. Questions will be answered and any confusion about the study will be resolved.

2) Assessment of capacity to consent: The staff will assess the potential intervention subject's capacity to consent using the UCSD Brief Assessment of Capacity to Consent form (UBACC) for the study. A score of 2 on items 1, 2, 4, 6, 7, and 9 is needed for inclusion in the study. If a participant scores less than 2 on any item, the staff will re-explain the study and reevaluate the capacity of the subject. Given the potential fluctuation of their cognitive capacity, if a subject still fails to score 2 on required items, we will ask the person to return on another day to re-take the UBACC. If the subject still scores below 2 on any required item during the 2nd visit, he/she cannot be enrolled in the study. Of note, other items than the required 6 items are provided for education purpose. If they do not answer 2 on those items, the staff will re-explain the study, but such failures will not interfere with their eligibility in the study.

3) The study partner subject signs the study partner subject consent for participating in the study after staff have reviewed the following study details with each study partner subject: 1) the objective of the project; (2) description of timeline, the assessment procedure and components; (3) description of the environment where the assessments are conducted; (4) description of how the confidentiality regarding participant data will be protected; (5) description of the potential risk, relevant protections, and benefits of participating in the study. Note: If the intervention subject's in-person screening appointment is scheduled to occur before the study partner subject's secondary screening appointment, and the intervention subject is deemed ineligible, the study partner subject will not be consented to the study.

## 8. STUDY PROCEDURES

**COVID-19 Safety Regulations:**

To ensure appropriate safety precautions when conducting in-person study procedures, the process for conducting in-person visits outlined in the Guidance for Human Subject Research webpage (https://www.urmc.rochester.edu/coronavirus/coronavirus-research/guidance-for-researchers/human-subjects-research.aspx) will be followed. We will remain vigilant about any further changes to the research reboot guidance.

This study will screen participants and their study partner subjects in two steps (phone-screening and secondary interview, including capacity to consent and informed consent). Eligible participants and their study partner subjects will be enrolled, and participants will be asked to complete a baseline cognitive assessment, MRI scan, and blood draw. The participant will then be randomized and start their intervention program within two weeks. Each program will consist of 14 tDCS sessions over 4 weeks. Follow-up cognitive assessments and MRI scans will occur at 4- and 8- weeks after baseline.

**Screening**: Potential participants who respond to our recruitment strategies and initiate contact with us will be carefully screened for eligibility and safety through a 2-step screening process:

(1) **Phone screening (approximately 20 minutes for intervention subject and partner subject**

**respectively)**: Upon over-the-phone contact, staff conducting the screening will explain the study using the phone screening script, answer any questions. If the participant is interested, the staff member will obtain their verbal consent to be administered the phone screening questions by signing and dating the verbal consent for screening form. Staff will collect basic self-reported health history to check for obvious contraindications for MRI or tDCS and to ensure a homogenous sample. Responses to these basic health history related items may need to be confirmed by their physician to ensure safety of MRI procedure are met. Identification of a "study partner subject" willing to participate in the study with the participant. Administration of the Neuropsychiatric Inventory Questionnaire (NPI-Q, a short form version of the NPI) with the study partner subject (after obtaining verbal consent from the study partner subject to be asked these questions) will also be conducted as part of this brief initial screening step,

**Secondary cognitive screening**: During the secondary screening interview (approximately 1.5 hours for intervention subject and 0.5 hour for partner subject), capacity to consent and informed consent will be obtained from the participant and their study partner subject. Study staff will verify the accuracy of phone screen data and note any changes, administer demographic and comprehensive health history questionnaires, administer measures to assesspresence of a clinical diagnosis of MCI (see **Table 1** for more information regarding measures used). Staff will also administer the full version of the Neuropsychiatric Inventory (NPI) with the study partner subject. Secondary screening should be completed within 3 months of phone screening. **Note:** For partner subjects, secondary screening may be completed over the phone, in which case informed consent and capacity to consent will be completed along with NPI. Once a study partner has passed the capacity to consent, blank versions of the consent form will be sent to the partner with a self-addressed, stamped return envelope, and be sent back signed. Coordinators will then verify partner signature, then sign and date each copy. A fully-executed copy of the consent form will then be sent back to the study partner. The intervention subject and study partner subject may complete their secondary screening appointments on separate dates if preferred.

We expect to invite 180-200 individuals for the secondary screening visit to enroll 40 fully eligible intervention subjects. 100-120 subjects are estimated to have signed consent forms but end up as screen failures or lose interest in the study.

**Assessments**: Fully eligible participants and their study partner subjects will be notified of their eligibility for the study. Baseline assessments need to be scheduled within one month of in-person cognitive screening. Participants will then be asked to complete baseline measures (assessment, MRI scan, and blood draw), after which they will be randomized to either the active or sham tDCS intervention condition. The blood draw may occur within 2 weeks (before or after) the baseline assessment and MRI scan. The beginning of the intervention phase will start within two weeks after the baseline assessment and blood draw. Follow-up cognitive assessments and MRI scans for each participant will occur within 2 weeks after completion of intervention (i.e., post-intervention assessment) and then 4 weeks after post-intervention assessment (i.e., follow-up assessment). We will allow 2 weeks to make up the follow-up assessment. At the same time points, study partner subjects will be administered the NPI, AES, and QOLAD. Follow-up assessments for study partners can also be conducted over the phone, at the partners discretion.

A detailed overview of data collection measures for screening and assessments can be found below in **Table 1**. To reduce the burden on participants, study partner subjects, and staff, as well as to avoid practice effects of cognitive measures, for individuals who are eligible for the study, their screening data will be used as baseline data; for those who are not eligible for the study, their screening data will only be used for summarizing the reasons for ineligibility.

Group assignment/randomization will be determined with a block-based matlab randomization.

**Table 1:** Study Measures I = intervention subject; P = partner subject

| | Screening | | Baseline | Interventions | | | | Makeup Interventions (if needed) | | Post-Intervention Assessments | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Study Activities** | **Phone** | **In-Person** | **BL Visit** | **Week 1** | **Week 2** | **Week 3** | **Week 4** | **Week 5** | **Week 6** | **Immediate Post-Intervention** | **Final Post-Intervention** |
| Verbal Consent for Screening | I, P | | | | | | | | | | |
| Inclusion/Exclusion (verified at In-Person) | I, P | I, P | | | | | | | | | |
| MRI Screening Form | I | | | | | | | | | | |
| Informed Consent | | I, P | | | | | | | | | |
| UBACC | | I | | | | | | | | | |
| Demographics & background | | I,P | | | | | | | | | |
| MoCA (V2) | | I | | | | | | | | | |
| RAVLT | | I (Lists C&D) | | | | | | | | I (Lists A&B) | I (Lists C&D) |
| ADL-PI-self | | I | | | | | | | | I | I |
| PSS | | I | | | | | | | | I | I |
| PSQI | | I | | | | | | | | I | I |
| MFI | | I | | | | | | | | I | I |
| GDS-15 | | I | | I | I | I | I | I | I | | |
| AES | | I, P | | | | | | | | I, P | I, P |
| QoL-AD | | I, P | | | | | | | | I, P | I, P |
| Medications | | I | | | | | | | | | |
| Medication changes | | | | | | | | | | I | I |
| NPI-Q | P | | | P | P | P | P | | | | |
| NPI (Full) | | P | | | | | | | | P | P |
| Examiner | | | I (Form A) | | | | | | | I (Form B) | I (Form C) |
| GDS-30 | | | I | | | | | | | I | I |
| C-SSRS | | | I | | | | | | | I | I |
| STAI | | | I | | | | | | | I | I |
| BVMT-R | | | I (Form 1) | | | | | | | I (Form 2) | I (Form 3) |
| DOSPERT | | | I | | | | | | | I | I |
| CRASH | | | I | | | | | | | | |
| Blood Draw with questionnaire | | | I | | | | | | | | |
| MRI scan (& confirm MRI screen form) | | | I | | | | | | | I | I |
| Intervention (sham or active) | | | I | I - One session per each weekday | | I - Two sessions per week | | I – Makeup sessions (if needed) | | | |
| MATLAB computer task | | | | I - During each intervention session | | | | | | | |
| SAM | | | | I - Before and after each intervention session | | | | | | | |
| Fatigue scale | | | | I - Before and after each intervention session | | | | | | | |

Note: For further details regarding measures, please see **Section 4: Study Design**.

**Blood collection and processing**:

We will collect a single blood sample from each participant within 2 weeks of the baseline assessment (before or after), and prior to beginning tDCS intervention sessions. This blood collection visit will require a separate appointment from the baseline assessment and MRI scan. Study staff will schedule the blood collection with CRC staff and confirm the appointment with the participant. Participants will be reminded to fast for 8 hours prior to their blood draw but may drink water and should take all medications as prescribed. On the day of the blood collection appointment, study staff will meet the participant at CRC and CRC staff will collect blood following a venous-blood collection protocol. Study staff will also ask each intervention subject 3-questions related to their diet and health in the past week. A total of 20mL of blood will be collected – 12mL into two 6mL plasma (EDTA-treated) tubes, and 8mL into two 4mL BD red serum tubes. Blood will be processed and frozen in cryo-vials at CRC -80° F freezer. Once cryo boxes are full (due to lack of space for storage of multiple cry-boxes in CRC) then cryo boxes will be transferred to 4W-156, bio-safety level lab 2 in School of Nursing for storage in -80° F freezer until analysis.

No individual research results will be provided to intervention subjects or study partner subjects in this study. However, aggregate group results will be disseminated to all subjects after study completion.

## 9. RISKS TO INTERVENTION SUBJECTS

The overall risks to participants for this study are expected to be minimal. In our feasibility study, no adverse effects were reported. The risks associated in the participant characteristics or study procedures include: (1) concern about suicide; (2) MRI; (3) tDCS; (4) blood draw.

**Concern and protection about suicide and harm to others:**

The assessment of suicidality or need for hospitalization will be based on following assessments throughout the study period: (1) responses to the C-SSRS at baseline and follow-up assessments. The C-SSRS assesses past and current risk of suicide and suicidal ideation. All coordinators in this study have been certified to administer this measure and obtain a risk score based on intervention subject responses. (2) GDS combined with P4 is assessed weekly during an in-person tDCS session, and follow-up assessment visits. GDS, specifically, responding 'No' to GDS item 11 – "It is wonderful to be alive now" will be followed immediately by the P4 suicidality screener, and by mental status examination of the participant. (3) the partner subject's response to the Depression/Dysphoria item #7 on the NPI. In the event that a suicidality risk is detected by the NPI, we will ask the partner subject about the risk of the intervention subject for hurting himself/herself, and administer to the P4 suicidality screener to the intervention subject within 24 hours. Across all three scenarios, the research staff will assess for severe hopelessness, passive death wish, suicidal statements, suicidal plan, or behavioral indicators of risk for self-harm. Need for hospitalization will be similarly assessed by a study physician. Hospitalization is typically indicated if there is imminent risk of harm due to agitation, such as refusal to eat, weight loss, violent behavior toward the caregiver, or suicidality. We have established a standard protocol for addressing concern on suicide patients at risk or with dementia, by working with certified geriatric psychiatrist or neurologist from our Memory Clinics (including Dr. Porsteinsson, co-I of the study and a geriatric psychiatrist working at the clinic). The clinician will provide immediate care to any participants at risk of suicide or clinically unstable. If a participant is

judged to be at immediate high risk and leaves study venue against the advice, we will contact security or other appropriate emergency personnel (e.g., ambulance, mobile crisis team).

For the partner subject's response to the Agitation/agression item #7 on the NPI, we have procedure in place for training staff's home visit to address the potential harm. If the staff detects an imminent risk of harm to the subject or others, the staff will call 911. Otherwise, the study team's geriatric psychiatrist and the PI will be contacted.

**Risk and Protection against Risk Related to MRI Imaging Assessments:**

There is no immediate risk from exposure to magnetic fields of 3 Tesla. Possible anxiety may result from claustrophobia or dizziness experienced by the intervention subjects when placed in the magnet. During the imaging portion of the experiment, intervention subjects must remain in the bore of the magnet, which is approximately 3 feet in diameter. Also, the scanning coil closely encloses the intervention subject's anatomy being imaged. These two factors may increase the likelihood of claustrophobia. Should the intervention subject feel discomfort, the experiment will be terminated upon their request.

In rare cases, contact with the MRI transmitting and receiving coil or conductive materials such as wires, or skin-to-skin contact that forms conductive loops, may result in excessive heating and burns during the experiment. The operators of the MRI scanner will take steps, such as using foam pads when necessary, to minimize these risks. The intervention subjects will be informed of the risk and instructed to immediately report any heating sensations. In the rare event that this would occur the experiment will be terminated and, if necessary, we will have the intervention subject seek medical treatment.

Intervention subjects will be screened for magnetic material before each study. Intervention subjects with pacemakers, aneurysm clips (metal clips on the wall of large artery), metallic prostheses (including heart valves and cochlear implants) or shrapnel fragments are at risk in an MR environment. Welders and metal workers are also at risk for injury because of possible small metal fragments in their eyes. Those at risk will be excluded from the study.

The effect of exposure to MRI scanning on an unborn child is unknown. Exposure to MRI scanning might be harmful to a pregnant female or an unborn child. There are no established risks at this time, but the intervention subjects will be informed that there is a possibility of a yet undiscovered pregnancy related risk.

MRI scanning produces a loud tone that can cause damage to the inner ear if appropriate protection is not used. Adequate protections in the form of earplugs or close-fitting silicon-padded headphones will be provided.

We cannot guarantee direct benefits from participating in the MRI assessment. fMRI are unique techniques in their ability to non-invasively study human brain function.

**Discovery of Previously Unknown Conditions** (conditions that can be diagnosed from having access to research-quality MRI):

Our scans are not read by a neuroradiologist, and the intervention subjects are explicitly told that the experiment will not provide information regarding their health status. However, if the certified MRI technician suspects something abnormal, he/she will seek advice from a qualified neuroradiologist, and the subject will be notified that his/her MRI indicated a potentially abnormal finding. With the subject's permission we will send information to their physician.

If a participant withdraws from the study, all attempts will be made to collect data to allow for inclusion in the analysis. Reasons for withdrawal will be recorded.

**Risk and Protection against Risk related to tDCS Conditions:**

The protocol described here uses stimulation levels that fall well within established safety limits. More than 500 research studies involving thousands of subjects have been published using tDCS (source: PubMed). A number of researchers have addressed the safety of tDCS. They have all concluded that tDCS is a painless technique for safely modulating cortical excitability. No serious or long-lasting effects have been reported. A recent meta-analysis of 209 studies found no serious adverse effects. The same meta-analysis found frequent mild adverse effects, such as itching and tingling[49]. All of these effects were transient and were localized to the skin at the stimulation site. A more focused study with 131 subjects undergoing 277 tDCS sessions reported similar findings: no serious adverse effects, but frequent mild effects, including itching and tingling[50]. The most common mild, localized, and transient adverse effects are, in order of decreasing frequency: itching, tingling, mild headache and burning sensation. Most people do not find these sensations to be painful. Skin redness in the area of stimulation is often reported. Itching, tingling and burning usually occur at the beginning of stimulation while the current is ramped up. These sensations are reduced by gradual current ramping (as it will be done in this study). Finally, some subjects report that these sensations are uncomfortable as some individual characteristics such as type of skin and hair might result in a higher stimulation of the skin nerve receptors. tDCS has only short lived, mild effects on brain activity. To ensure safe use of the tDCS device, the device will be carefully inspected before each use. The batteries will be removed after each use to prevent corrosion that could occur after long periods of non-use. The device will be stored in a dry location. Of note, in our pilot study of older subjects (reported in the "preliminary studies" section), there have been no adverse events.

We will provide training of the stimulation to our experienced project coordinators. The training will be provided by co-I Dr. Tadin, who is an expert in tDCS, and Mia Anthony (an experienced tDCS coordinator working on our feasibility study). Evaluation will be conducted by investigator team before a project coordinator can independently work on it.

The interventionists will ask every intervention subject's study partner subject to complete the NPI-Q weekly during the intervention period to monitor changes in NPS among the intervention subjects during the 4-week tDCS sessions. If any symptoms worsen over the intervention period and a worsening is confirmed by the study partner subject, it will be reported as an adverse event (in accordance with reporting procedures described in Section 16). The intervention will then be discontinued. Our co-I, Dr. Anton Porsteinsson, who is a geriatric psychiatrist, will be notified and provide medical assessment and further advice for medical assistance.

**Device-Specific Risk Minimization:**

Soterix Medical outlines the same risks and risk control features for both devices, **tES Model 2001** and tDCS Model 1300A, as follows:

**Irritation**

- The applied current may cause minor irritation, discomfort, and/or and redness at the electrode sites.
- Soterix Medical *EASYpad*™ electrodes should not be placed over previously irritated, burnt, or damaged skin.

**Methods of Risk Minimization:**

- **SMARTscan**™: a visual 20 bar LED display (1 to 20 from left to right) that indicates to the

operator the contact conditions of the electrodes before, during, and after stimulation. LED 1 denotes short condition and LED 2 denotes open-circuit condition. Stimulation should not begin if either LED 1 or LED 2 is lit.

- **PRE-STIM TICKLE**: a pre-stimulation feature that conditions the skin prior to full stimulation,
- **RELAX:** a feature that allows the operator to decrease the set level of current from the maximum (FULL CURRENT) value to accommodate subject feedback at any point without aborting the stimulation session.
- **TRUE CURRENT™** display is active whenever the device is on and indicates the actual value of current (in mA) being supplied from the device to the electrodes, regardless of device settings. This feature functions as a fully independent and redundant safety feature when monitored by the operator.

- **Stimulation ABORT**: this feature allows the operator to terminate the stimulation at any point by pressing the ABORT button, which *will ramp down the current to zero in 30seconds and terminate the entire stimulation run. Of note, too rapid ramp down (< 10 seconds) are not recommended.* Aborting the stimulation run is recommended if the TRUE CURRENT™ display deviates from expected output current, the TIME REMAINING display deviates from expected duration setting, intervention subject complaints any unusual adverse effects (i.e., beyond itching, tingling, mild headache, and mild burning sensation), and/or request to stop. In the event that the stimulation is terminated, the device will not be re-started and the session will be considered complete.

**Risk and Protection against Risk Related to Blood Collection:**

We will explain to intervention subjects the potential risks from venous blood collection, which include pain, a bruise at the point where the blood is taken, redness and swelling of the vein, infection, and a rare risk of fainting. We will utilize trained CRC staff to implement the following strategies to reduce the potential risks associated with venous blood collection and to protect research intervention subjects. To reduce pain, the CRC staff will use a needle of smaller gauge than the selected vein. To prevent bruising, CRC staff will insert the needle into the selected vein at an angle of 30 degrees or less. In addition, the use of a needle of smaller gauge helps prevent bruising. For the rare risk of fainting, intervention subjects will be asked to lie down if they express concern. Snacks will be provided to intervention subjects after each blood collection.

**Other Procedure-related Considerations for Protection Against Risks**

**Informed Consent:** At the screening stage, we ask the intervention subject for verbal consent for phone screening. If eligible to continue with step 2 of screening, decision-making capacity will be assessed using San Diego Brief Assessment of Capacity to Consent (UBACC) to ensure older adults who attend the proposed study have adequate capacity for giving consent and making decisions prior to enrollment (see section 7 for informed consent details).

Additionally, intervention subjects who lack capacity to consent may become emotionally distressed by that determination. However, intervention subjects will be reminded that this determination is merely meant to ensure their continued safety and understanding of the research study only, and does not indicate any impairment in their daily lives (see additional protections against risks for more details).

**Psychological Distress**: Although the questions asked of the intervention subjects and study partner subjects involved in the study have been regularly used in RCTs, the challenges from answering questions about another person, or knowing a study partner subject will be asked to answer questions about the intervention subject themselves, may produce frustration or anxiety. The PI will work closely with the research staff and intervention subjects/study partner subjects to help alleviate anyconcerns.

**Confidentiality and Privacy:**

Intervention subject data will be collected with the verbal and/or written consent of the intervention subject and study partner subject. Information pertaining to individual participants will be released with the participant's permission only. All participant data will be identified by a uniquely coded screening ID assigned to each respondent and study ID for participants eligible for enrollment. Access to the master

links between name and screening ID, as well as between screening ID and study ID, will be restricted to the study team.

All hard-copy based materials and data will be kept in a locked research office in a locked file cabinet. No one other than the study team will have access to the data. Once data have been entered into a REDCap Consortium (Research Electronic Data Capture), only code numbers (not names) will be associated with individuals' research data records. REDCap is a free, secure, HIPAA-compliant, web-based application used for electronic management of research study data, which can then be downloaded into SPSS for data analysis. REDCap servers are housed at the University of Rochester and all web-based information transmission is encrypted. Data from the REDCap database will be directly downloaded to a password-protected UR-SON server for access by the PI and study team. A list of names, home addresses, email (optional), telephone numbers of study participants will be used only for study purpose, and study management software (Filemaker Pro) will be used by the study team only, in a secure manner on UR-SON servers. All School of Nursing servers are routinely audited for vulnerabilities or improper configurations by the University of Rochester's Information Security team using the Nessus scanner, which is an industry leader in security assessment.

## 10. POTENTIAL BENEFITS TO INTERVENTION SUBJECTS

Non-invasive brain stimulation has the potential modify cognitive and affective status for older adults at risk for dementia but this has not yet been proven. The relatively modest risks associated with the activity are reasonable in light of its potential benefits. Participants will have the opportunity to receive a 4-week guided and supervised tDCS sessions (including visual attention task for each tDCS session) and interact with the study staff.

## 11. COSTS FOR PARTICIPATION

No

## 12. PAYMENT FOR PARTICIPATION

Participants will be compensated for up to $170 for the entire study, including $50 for each assessment, and $20 for the blood draw. Study partner subjects will be compensated for up to $60 for the entire study, including $10 for each NPI completed at each of the 4 weeks of the intervention and post-intervention and follow-up assessments. We believe this is a reasonable amount for the time and effort a participant and their study partner subject will contribute to the study. In addition, participants will be providing reimbursements if they incur parking or transportation costs when attending study-related activities.

## 13. SUBJECT WITHDRAWALS

Participants may be excluded from the study without their consent or withdraw from the study at their own discretion.

Withdrawal Circumstances: Anticipated circumstances under which participants may be excluded from the research without their consent include:

- Medical conditions which make ongoing participation in the study unsafe for a participant (e.g., change in MRI eligibility, seizures, severe depression, and/or suicidal ideations)

- If the intervention subject withdraws then the study partner subject is automatically withdrawn

## 14. PRIVACY AND CONFIDENTIALITY OF SUBJECTS AND RESEARCH DATA

Data Security: Participant data will be collected with the verbal and/or written consent of the participant. Information pertaining to individual participants will be released with the participant's permission only. All participant data will be identified by a uniquely coded screening ID assigned to each respondent and study ID for participants eligible for enrollment. Access to the master links between name and screening ID as well as between screening ID and study ID will be restricted to the study coordinator, interventionist, and staff involved in screening.

Only trained study personnel will have access to participants' research materials, which will be kept in locked facilities and password protected computer system. Manuals for training staff on different parts of the data collection have already been developed for different studies, which will be assembled for the proposed study.

All participant data (including those collected at the screening) will be collected with the verbal or written consent of the participant and study partner subject. Information pertaining to individual participants will be released with the participant's informed and written consent only. Participant data will be identified by a uniquely assigned study number. Access to the master list of study numbers will be restricted to the PI and study staff and maintained and stored in a lab database (Filemaker, Filemaker Inc.) on Filemaker server at the URMC. The primary source of study data (computer- and questionnaire-based data) will be stored at the lab database (SPSS and Excel). All of these databases are located at a secured site, coordinated through the Medical Center at the University of Rochester. Access to these databases is restricted to the investigators and trained study personnel. Publications or presentations will report only cumulative data or descriptions certain to maintain participants' anonymity. We understand that these data are subject to the Privacy Act, Freedom of Information Act, and other Federal government rules and regulations, and we will comply with those rules and regulations.

None of the information collected in the study will be automatically recorded to EHR since they are all collected in research settings.

For intervention subjects with suspected brain changes identified by CABIN appointed radiologist/neurologist (informed by CABIN technician), we will communicate this information with the subjects, and send them a picture from T1 upon signing the MRI release form. It is the subject's responsibility to share the form with their healthcare providers.

## 15. DATA AND SAMPLE STORAGE

**Blood Specimen:** Blood samples will be collected & processed at CRC. Full cryo boxes will then be transferred to 4W-156, -80 freezer in the SON until analysis is completed. Blood Sample Data: We will collect information on the following 8 biomarkers: Aβ42, t-tau, p-tau, NfL, BDNF, IGF-1, APOE 4, and 2 SCACs [C0 and C5]. In addition, we will measure other blood-based biomarkers such as phospholipids. All data will be stored on an encrypted, password-protected database on the URMC server that is only available to study staff. Individual databases used to house identifying subject information (telephone number, address, date of birth) will also be stored on the URMC server, on a password-protected file management system, accessible only to study staff. Both databases will be managed by the UR information analyst on site. All datasets will be stored pursuant to IRB protocols.

## 16. DATA AND SAFETY MONITORING PLAN

We propose a Stage 0 pilot intervention study. The study risk is minimal. The safety monitoring of which will be conducted by the research team. All investigators will compose an administrative core to oversee the project, holding regular monthly meeting with the research team. For each monthly meeting, the PI will present an overall progress statement. The issues/concerns related to the safety of participants will include:

(1) The evidence of safety issues that should be addressed;

(2) All serious adverse events determine whether individual patients should be removed from the protocol. We acknowledge that there may be rare instances where some emergent situation occurs that was unanticipated regarding the welfare of the participant. In these situations, the UR IRB may be contacted to help resolve the situation.

In addition, the following steps will be taken to protect the confidentiality of data and computer records and participant safety:

(1) Confidentiality will be assured by the maintenance of the data forms in locked offices, and by restricted access to computerized data. Only core research team members will have access to the name, address, telephone number, and other information corresponding to each identification number. Participants will be fully informed of the study requirements throughout the conduct of the study and will be allowed the opportunity to withdraw from participation if they cannot, or do not comply with the rigors of the research protocol. Handling of data will be limited to the numerical values and statistical summaries.

(2) Identifiers linking identification codes with individual names will only be available to the PI and study staff who contacts participants.

(3) The PI has obtained the policies of the UR IRB specifically regarding adverse events associated with the study. The PI will adhere to those policies and maintain a copy of the policies in the study file.

(4) The investigators will protect the health and safety of participants, inform them of information relevant to their continued participation (e.g., newsletters) and pursue the research objective with scientific diligence.

(5) The following policies required by our IRB and NIH will be adhered to: (1) any adverse events that are serious and unexpected and are related (possibly or probably) to the study will be reported to the IRB and NIH within 15 calendar days; (2) adverse events that are both unexpected and related that are either life threatening or result in death will be reported to IRB and NIH immediately; and (3) for adverse events that do not meet the criteria above will be documented in the summary report submitted to the IRB and NIH annually at the time of the study's continuing review. Because the proposed study is minimal risk, we do not anticipate any serious adverse effects as described in the first two categories from a result of participating in this study.

**Expected Adverse Events**:

- Temporary tingling or itching sensation from tDCS.
- Dizziness, mild nausea, headache, a metallic taste in their mouth, sensations of flashing lights, heating, and risk of metal projectiles during MRI.
  - Rating criteria for the severity of adverse events:
    1 = Mild event, no treatment required;
    2 = Moderate event, resolved with treatment;
    3 = Severe event, resulted in inability to carry on normal activities, ongoing medical treatment required;
    4 = Life threatening or fatal.
  - Attribution of the adverse event to the study:
    Non-study related = Event clearly not related to doubtfully related to the study;

Study-related = Event possibly, likely, or clearly related to the study.

(6) The PI will ensure that the NIH (funding Institute and Center) is informed of the actions, if any, taken by the IRB as a result of its continuing review, and recommendations that emanate from the monitoring activities.

## 17. DATA ANALYSIS PLAN

This is a pilot study for mechanistic understanding of the relationship between our previously discovered NPS-shared neural circuit and study partner-rated NPS. Results from the study will provide information for accurately calculating effect size for future clinical trial. Regardless, in addition to follow the rule of 12-20 subjects per group for a pilot clinical trial, we will calculate the sample size. The sample size calculation is based on the effect size of LPG related tDCS on visual attention performance (f = 0.45, n = 36). Additionally, the effect size of correlation between NPS-shared neural circuit and patient-report NPI-Q was $r^2 = 0.70$ in our previous study[3] – here we use a much more conservative estimation at $r^2 = 0.20$, the sample size will be n = 37. All of these calculations are based on power at 0.80 and alpha at 0.05. For an 8-week follow up, we estimate 10% attrition rate. Therefore, we propose a total of 40 intervention subjects for the study.

**Aim 1. Determine the effect of tDCS on NPS-shared neural circuit**. **H: After intervention, the active, relative to sham group will have greater increase in LPG activity, indexed by greater activation in LPG in response to visual attention task (H1a) and greater increase in resting FC strength between LPG and other regions within the NPS-shared neural circuit (H1b).** To assess the activation pattern in LPG, we will conduct GLM analysis with the SPM software. The coefficients of GLM corresponding to LPG will be analyzed through hypothesis testing. We will first conduct a simple t-test to evaluate whether the intervention increases LPG activation for each group (at 4 or 8 weeks), and then a more powerful two sample kernel test will be used to test activations distribution difference between groups.[51] Similarly, we will take the FCs between LPG and other regions as a multivariate covariate and apply the two-sample kernel test to evaluate group difference in the change of LPG FC pattern after intervention (at 4 or 8 weeks). In addition, simple multiple testing for each connection will be used as a screening procedure to identify a subset of connections included in the kernel test.

**Aim 2. Determine the relationship between NPS-shared neural circuit and study partner-report NPS.**

**H2a. After intervention, the active, relative to sham group will have greater improvement in study partner-rated NPS in terms of lower severity and fewer co-existing symptoms:** We will fit linear mixed-effect models for changes in NPS: $Y_i = X_i\beta + Z_i u_i + \epsilon_i$, where $Y_i = (y_{i1}, \ldots, y_{iT})$ represents the NPI measures across time, $X_i$ is a $T \times p$ design matrix for the fixed effects and $Z_i$ is a $T \times q$ matrix for the random effects. The models will include fixed effects such as data collection visit (categorical variable), group, visit and group interaction, and covariates identified as being imbalanced among groups. Stepwise variable selection based on Akaike Information Criteria will be used to select the fixed effects for inclusion. A random participant-specific effect will be included to account for correlation between visits for the same participant. The model-based average within-group change will be computed for each outcome at 4 and 8 weeks. This will allow us to rank the groups in terms of average within-person gains in each outcome. We will conduct a composite hypothesis test to assess if the changes at 4 or 8 weeks differ by groups (similar to a one-factor ANOVA but accounting for repeated measures). **H2b. FC change in NPS-shared neural circuit will contribute to the improvement in study partner-rated NPS across groups:** We will perform epsilon-intensive support vector regression (SVR) with RBF kernel using LIBSVM library.[52] The Leave-One-Out-Cross-Validation scheme will be applied: each intervention subject will be designated as

the test sample once, while the remaining intervention subjects will be used as training sample. All training sample's features (change of voxel activation from the task or change of FCs from the NPS-shared neural circuit after intervention) will be trained to predict the test sample's outcome (change of NPI severity or frequency score). We will then perform model fitting of the raw and predicted NPI using linear function ($f(x)=ax+b$). Adjusted $R^2$ will be used to estimate goodness of fit for the model.

**Exploratory aim. Examine the relationship between (a) NPS and (b) the coherence between structural and functional aspects of the NPS-shared neural circuit**. We will conduct a *connectome-based analysis* to find structural connections that are unique to severity of NPI. The TN-PCA method[53] will be used to correlate with severity of NPI. We can replace the high dimensional tensor network summarizing an individual's brain connectome with a K-dimensional vector of brain PC scores; these scores can be used for visualizing variation among individuals in their brain connectomes and in statistical analyses studying relationships between structural and functional aspects of NPS-shared circuit and covariates. We will compare similarities of involved brain regions of NPS circuit for FC and WM across the entire sample at baseline. Quantitatively, we will use the Dice similarity coefficient to evaluate the percentage of overlapped regions. **To examine whether tDCS' effects on NPS-shared neural circuit and study partner-rated NPS depend on baseline WM integrity,** first we will compare the correlations between FC (at different time points) and baseline WM integrity on the overlapped regions between active vs. sham tDCS groups. We expect to see a higher correlation between FC and WM in active group in later time points. Next, using SVR described earlier[52] we will examine the relationship between the sample's features (baseline WM tracts) and outcome (change of NPI severity or frequency scores).

**Blood analysis plan:** We will compare AD pathology markers from blood with changes of NPS, cognitive measures, and MRI data using correlational analyses. We will also examine if individuals with positive AD pathology respond differently to tDCS compared to those with negative AD pathology using ANOVA.

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