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PART B
STUDY DESCRIPTION

TITLE OF PROTOCOL	**The Development and Human Translation of Temporal Interference Brain Stimulation**
Principal Investigator	**Daniel Press, MD**

B1. PURPOSE OF PROTOCOL

The primary aim of this study is to translate temporal interference (TI) stimulation methodology into humans and examine its safety, feasibility, steerability, and focality. In the proposed early phase human experiment, we will assess the ability to apply TI stimulation along spatial dimensions to selectively modulate neural activity and assess the feasibility of selective targeting deep brain structures without exciting overlaying cortex. The overall goal of the study is to advance TI methodology and its translation to humans.

The specific aims in this study are to

- Assess the safety of TI stimulation.
- Assess the feasibility, focality, and steerability of TI stimulation by selectively modulating activity in subregions of a cortical area (calcarine cortex)

We hypothesize that TI stimulation can be used to impact different regions of the visual field that are represented within the calcarine fissure of the human brain.

We hypothesize that TI stimulation is possible in regions within the calcarine fissure without impacting intervening regions.

We hypothesize that TI will be well tolerated by human subjects and side effects will be consistent with other forms of transcranial electric current stimulation (tES).

B2. SIGNIFICANCE AND BACKGROUND FOR THE STUDY

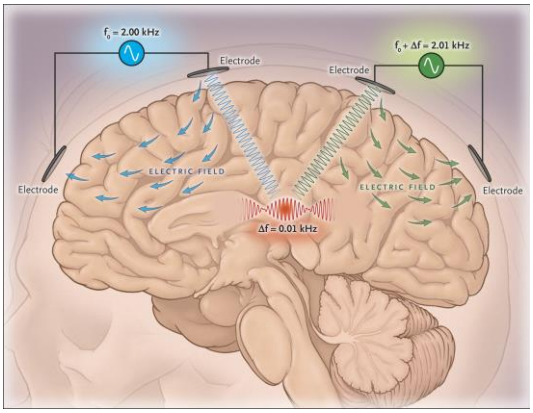
<u>Current brain stimulation methods have limitations:</u>

Deep brain stimulation (DBS) has had great impact in disorders such as Parkinson's disease and obsessive-compulsive disorder and has potential for other disorders such as depression and Alzheimer's disease. DBS works by delivering focal electrical stimulation from electrodes surgically implanted in deep, often sub-cortical regions. Being a surgical procedure, it bears the potential for serious complications that limit adoption and scalability.

Transient non-invasive brain stimulation technologies, such as transcranial magnetic stimulation (TMS) and transcranial electrical stimulation (tES), have shown efficacy as a therapeutic option for treatment resistant depression and are being investigated for stroke rehabilitation and many other brain disorders. However, the ability of TMS or tES to directly stimulate deeper brain structures is obtained at the expense of inducing stronger stimulations of overlying cortical areas, and the resulting wider stimulation often will push on the limits of safety guidelines. Recently, transcranial focal ultrasound has been shown to modulate deep brain regions, but the mechanism is currently unknown, and some results suggest cellular damage might result. There is still great hope for a method of brain stimulation that could be focal, steerable, selectively deep, and electrical.

Temporal interference (TI) stimulation:

We recently discovered a strategy for sculpting the amplitude of electric fields so as to enable focal, yet noninvasive, electrical neural stimulation. We discovered that by delivering multiple electric fields to the brain at slightly different carrier frequencies — which are themselves too high to recruit effective neural firing, but for which the offset frequency is low enough to drive neural activity — we can cause neurons to be electrically activated at a focus without driving neighboring or overlying regions. We call this method temporal interference (TI) stimulation, since the interference of multiple electric fields is what enables the focality: neural stimulation will occur only at the region for which the amplitude of the electric field envelope, at the offset frequency, is of great magnitude. We examined whether the TI concept could indeed result in well-defined low frequency envelope modulated electric fields through computational modeling using finite element methodologies (FEM), and found that indeed we could create an electric field with peak low frequency envelope at a deep point within a conductive structure, without significant low frequency electric field at overlying areas. (See figure)



TI stimulation cartoon. Two alternating current sources are placed on the head. Where the two fields converge, a low frequency envelope electrical field occurs. (Figure 1, from Lozano 2018, NEJM)

We here explore and develop this physical principle to make it relevant to the brain, which has not been done before. One particularly appealing reason for exploring TI stimulation is the very solid scientific premise: it is based upon the laws of physics, and easily validated by computational modeling and physics experiments. However, the precise mechanism is not understood, and a key goal of our study will be to understand the mechanism in terms of bioelectric effects within and between neurons in neural networks.

Animal studies:

We validated that such interferometrically generated low frequencies could effectively drive neural activity by applying the TI stimulation strategy transcranially to anesthetized living mice, and recorded the responses using whole-cell patch clamp in current clamp mode, using our autopatching robot, finding that indeed neurons would follow the low frequency envelope of the electric field, but not the high frequency carrier.

To test whether the TI stimulation methodology could be used to recruit deep brain structures without recruiting overlying neural layers, we transcranially applied TI stimulation and control stimulation conditions to the hippocampus of anesthetized living mice and measured the activation profile using c-fos staining. We found that 10 Hz transcranial stimulation resulted in very broad c-fos expression profiles, with a strong activation in the cortex as well as in the hippocampus. In contrast, transcranial application of 2 kHz + 2.01 kHz TI stimulation, with an envelope amplitude peak deeper than the cortex, resulted in c-fos expression only in the hippocampus with no significant neural activation in the cortex. We found that the TI stimulation did not alter the neuronal and synaptic integrity of the underlying tissue 24 h after stimulation, at least as reflected by the stains we used.

To test whether TI stimulation could evoke behaviorally relevant responses, we stimulated the forelimb region of the motor cortex and measured evoked motor activity. We found that simultaneous application of currents I_1 and I_2 at frequencies $f_1 = f_2 = 2\ kHz$ did not evoke motor activity. However, when f_2 was set to 2.001 kHz, 2.005 kHz or 2.01 kHz, while keeping f_1 fixed at 2 kHz, the stimulation evoked a movement of the contralateral forelimb at the difference frequency of 1 Hz, 5 Hz and 10 Hz, respectively. To test whether the evoked movement could be steered without physically moving electrodes, we increased the current I_1 above the forelimb area of the motor cortex and decreased the current I_2 above the whisker area of the contralateral motor cortex, keeping the current sum fixed (predicting steering toward the I_2 electrodes). We found that the movement amplitude of the contralateral paw decreased and the movement amplitude of the contralateral whisker increased. When I_1 was larger than I_2, the stimulation evoked a movement in the whisker ipsilateral to the I_1 electrodes, with stronger movements evoked as the amplitude of I_1 increased compared to I_2. This suggest that the steering anticipated in our modelling can be realized in-vivo.

Human pilot testing:

Researchers at MIT have completed relevant pilot testing to assess whether TI stimulation is safe and can recruit activity in the human brain. We applied TI stimulation to the motor cortex of nine healthy volunteers (three females; mean age 27.5 ±5) and measured BOLD signals concurrently using fMRI. The experiment included four sessions consisting of six stimulation blocks - 10 Hz stimulation, 2 kHz stimulation, TI stimulation with 1:1 current ratio, TI stimulation with 1:3 current ratio, TI stimulation with 3:1 current ratio, and sham. Each stimulation block lasted 25 s with 5 s ramp-up and ramp-down periods. All participants tolerated the procedure without any adverse or side effects. No pain, tingling, cognitive changes, or sensory-motor difficulties were reported for the TI case by any of the participants. To test whether the TI stimulation changed the BOLD signal at the brain regions that were exposed to the electric fields we compared the T-score inside a spherical region of interest (ROI) in which activation was predicted by a subject-specific FEM modeling. We found that TI stimulation with a current ratio of 1:1 increased the BOLD signal at the center of the ROI. In contrast, TI stimulation with a current ratio of 1:3 increased the BOLD signal primarily at the frontal half of the spherical ROI, while TI stimulation with a current ratio of 3:1 increased the BOLD signal primarily at the dorsal half of the ROI, suggesting that steering might be possible in humans.

TI Validation with Visual Field Stimulation:
The organization of the human visual system is an ideal model system suited for evaluation of the steerability of TI stimulation. The primary methodological advancement of TI is stimulation of deeper structures leaving superficial structures near the electrodes unaffected. The human primary visual system is organized “retinotopically”, that is, with respect to the center of gaze. It is located within the calcarine sulcus, located in the occipital lobe at the posterior of the head. The most posterior part of the sulcus represents the center of gaze, and the most anterior part of the sulcus represents the periphery. Using this feature, we will examine if stimulation targeting the anterior calcarine sulcus induces a detectable visual change within the peripheral visual field, without impacting the center of gaze, located more superficially. Additional control sessions will evaluate the precision of TI steering.

Visual stimulation has historically been used as a preliminary investigation of non-invasive stimulation, in the study of transcranial magnetic stimulation (TMS) and transcranial electrical stimulation (tES). These sessions in healthy participants have shown acute effects of stimulation (e.g. brief phosphenes, transient scotomas) that are transient.

Clinical significance:

The importance of the problem we are tackling with TI stimulation is enormous, clinically speaking.

Approximately 100,000 people have had DBS implants, requiring invasive surgery, but the patient population that could be helped is potentially in the many millions – and noninvasive stimulation could potentially help. While DBS has proven effective for some patients, one critical barrier for its wider therapeutic impact is eliminating the serious complications that can result from surgery and making the effects more widely available than can be deployed by the relatively small number of neurosurgeons. Currently, perhaps 1% of the current tremor population receives DBS due to the high risk involved; by making DBS noninvasive, we will help increase the accessibility of this kind of treatment by perhaps orders of magnitude. On the other hand, noninvasive brain stimulation methods, such as TMS or tCS, could potentially help many more people if we were able to make it more steerable and more focal. Thousands of studies have been performed with noninvasive brain stimulation, and four different TMS devices have been approved for the treatment of medication-resistant depression[18], raising the question of whether having better focality, precise steerability, and depth selectivity, might open up TMS/tCS-like protocols to broader patient populations as well.

The key goal of this proposal is to advance the translation of the TI stimulation methodology to humans. At present, TI stimulation cannot match the spatial resolution of implantable DBS at depth. We hypothesize that in the future it might be possible to pinpoint smaller regions of the brain using a larger number of interfering kHz-frequency electric fields, contingent on a similar neural response to those individual fields. TI stimulation has also not been tried with different frequencies (e.g., neural drive frequencies) as high as those used in DBS. We will explore these higher frequencies, e.g. at 100 Hz and beyond, in this proposal.

In conclusion, the proposed project will offer new experimental frontiers to map and modulate the brain with a non-invasive alternative to DBS/TMS/tCS with low risk, precise steerable focality, and depth selectivity. We will advance the TI methodology and initiate its translation to humans.

B3. DESCRIPTION OF RESEARCH PROTOCOL

A. Study Design – Overview, Methods, Procedures

Overview

This is an investigational early phase testing of temporal interference (TI) stimulation in humans. The overall aim of the study is to assess the safety, feasibility, focality, and steerability of TI stimulation by selectively modulating activity in subregions of a cortical area (calcarine cortex – the primary visual cortex)

Healthy subjects who meet inclusion and exclusion criteria will be entered into the study. The study will recruit up to 20 subjects with the aim to complete 12 subjects.

Study Visits:

The study will consist of up to 6 study visits. The screening and baseline visit, the MRI visit, and up to 4 TI study. The screening and baseline visit and TI visits will occur at Beth Israel Deaconess Medical Center in the Berenson-Allen Center. The MRI visit will take place at the Boston University Cognitive Neuroimaging Center.

- ***Screening and Baseline visit (Visit 1):***
  Subjects will be prescreened over the phone or REDCap and invited in to the Berenson Allen Center to complete screening and baseline if they appear eligible and continue to be interested in the study. After Informed Consent is obtained, the following screening and baseline procedures will be completed:

  - Inclusion and exclusion criteria review
  - Subject demographics
  - Handedness assessment
  - Medical history and medication review
  - Physical and Neurological exam conducted by a Neurologist or Neurologic Nurse Practitioner
  - Baseline perimetry assessment (may be completed during/after MRI visit at BU)
  - The MINI International Psychiatric Interview Psychiatric assessment
  - All female subjects will undergo a pregnancy test and pregnant women will be excluded
  - Screening for retinotropic mapping – assessing the participant's ability to hold fixation with their eyes for experimental trials (may be completed during/after MRI visit at BU)
  - MRI safety review

  The baseline and screening visit will take approximately 1.5 hours.

- ***MRI session(Visit 2)***
  The MRI session will take place at the Boston University Cognitive Neuroimaging Center under a Boston University submitted and approved protocol that is specific to this study. An MRI scan of the brain will be conducted while the participant views visual stimuli to obtain each individual's retinotopic map. This data will be provided to the study team at BIDMC to conduct the study visit and for analysis (Further details in "Data Security")

  This visit will take approximately 1.5-2 hours.

- ***Temporal Interference (TI) Sessions (Visits #3-7):***

Each subject will then undergo up to 4 TI stimulation sessions (2 minimum) separated by at least 2 days to minimize the risk of carry over effects of the stimulation. In each visit, the participant will receive TI stimulation to a region of retinotopic representation in the calcarine fissure. The cortical targets will be defined by electrical field modelling that will be used to optimize the electrode placement.

Stimulation visits will consist of TI stimulation combined with a visual discrimination task, with any changes in visual perception assessed by collecting behavioural responses (i.e. button presses) from the subject in response to visual stimuli presented before, during, and after TI stimulation.

Each visit will consist of up to four, 10 minute blocks of stimulation paired with a visual discrimination task. The stimulation blocks will be completed with TI stimulation at a difference frequency ranging from 1 to 80Hz, transcranial alternative current stimulation (tACS) stimulation with no temporal interference at a frequency ranging from 1 – 80HZ, or a no offset stimulation (e.g. matched carrier stimulation frequencies such that no envelope modulation is anticipated). The most common signal from visual cortex during wakeful relaxation is in the α frequency range (8-12 Hz). Due to this feature of visual cortex, we hypothesize that stimulation frequencies in and around α are likely to demonstrate TI efficacy. Moreover, since visual perception changes have been induced with tACS at 60Hz, we hypothesize that stimulation in and around this frequency will associate with detectable visual perception changes. We hypothesize that TI with a residual effective stimulation frequency of 1-80 Hz will be ideally suited for activation of the targeted visual cortex.

Each block will go as follows:
1. Baseline visual discrimination task to assess threshold
2. TI stimulation paired with the visual discrimination task and visual disturbance assessment (10 minutes)
3. Repeat visual discrimination task

Participants will have a 5 minute break between blocks.

Participants will be monitored throughout the visit for any adverse effects and a tES side-effect questionnaire will be administered at the beginning and end of each stimulation visit to additionally track any adverse effects. Although any visual disruption induced by the stimulation is expected and anticipated to be transient in nature, at the conclusion of each visit, a standard automated visual perimetry assessment will be completed on a Humphrey Visual Field analyser to compare to baseline. If there is a change in the perimetry assessment, a study MD will evaluate the participant to determine the need for additional follow-up. If there are any immediate concerns, the participant will be further evaluated by the study ophthalmologist. Overall, each stimulation visit will take approximately 3 hours to complete.

Detailed Methods and Procedures:

Mini International Neuropsychiatric Interview (MINI):
All subjects will be screened as overseen by a board-certified psychiatrist using the Mini International Neuropsychiatric Interview, ver. 6.0 (MINI) to identify prospective subjects with exclusion criteria related to mental health.

If a subject answers affirmatively to the question *"Did you repeatedly consider hurting yourself, feel suicidal, or wish that you were dead? Did you attempt suicide or plan a suicide?"* within the past 2 weeks, a study MD (psychiatrist or neurologist) will be notified and will assess the participant/the participant's

responses. The MD may recommend follow-up with their primary care provider and/or with a mental health care provider. Although not anticipated in this study of healthy individuals, if a participant is thought to need urgent care, the participant's healthcare provider may be contacted by one of the study clinicians to develop a plan for follow-up. If there is concern for their immediate safety, they will be sent to the emergency room at BIDMC.

MRI

The subject will go to Boston University Cognitive Neuroimaging Center and will have the MRI under a Boston University submitted and approved protocol The MRI will include T1 and T2 structural images. Retinotopic mapping and population receptive field mapping (pRF) will be performed using T2* weighted EPI (BOLD) images. Participants will view a computer screen projected within the bore of the MRI. The computer display will consist of visual stimuli presented in regions of the visual field to evoke targeted visual responses in the brain. Participants will be asked to make button presses to specific stimuli within the display while they keep their eyes still. Individual MRI runs will be 4-12 minutes, giving brief breaks to participants between runs for the whole MRI session.

Although the scan will be collected under a separate protocol at Boston University, the data will be sent to BIDMC and the study team at BU will alert the BIDMC PI if they note any findings to be reviewed. Any suspected incidental findings will be reviewed by a neurologist, who will share the findings with the patient in real-time and will recommend follow-up with their primary care provider and/or will recommend that they follow-up with a specialist provider. Although not anticipated in this study of healthy individuals, if a participant is thought to need urgent attention, the participant's healthcare provider may be contacted by one of the study clinicians to develop a plan for follow-up.

Visual field Testing

All participants will have a baseline visual field assessment with a computerized visual field device (a Humphrey field analyzer – HFA) to be completed in the BIDMC ophthalmology department. This assessment will also be completed at the end of each TI visit. The assessment consists of the participant being positioned against a forehead and chinrest while being asked to maintain fixation on a central target and while providing responses when a light stimulus is noted. One eye will be assessed at a time. The testing takes under 10 minutes. If an abnormality is noted at baseline, the study ophthalmologist will review the results and will share the findings with the patient in real-time and will recommend follow-up with their primary care provider and/or will recommend that they follow-up with a specialist provider in ophthalmology. They will be excluded from the study if an abnormality is discovered at baseline. If a change is noted at the end of a testing session, a study MD will evaluate the participant to determine the need for additional follow-up. If there are any immediate concerns, the participant will be further evaluated by the study ophthalmologist who will provide recommendations for observation or follow-up.

Visual Discrimination and Disruption Testing

Participants will be asked to perform a visual discrimination task within the targeted region of visual space and within a control region prior to, concurrently, and following TI stimulation.

Adaptive thresholds will be computed for orientation discrimination and contrast discrimination using an algorithmic approach. Discrimination thresholds for stimulated and unstimulated regions will be assessed using a within-subjects design using a repeated-measures ANOVA. Subjects will be asked to fixate at different locations on a screen and press a button, for example, to indicate where they see a visual stimulus.

TI Stimulation

TI stimulation will be generated by our prototype device and applied to the head via standard, carbon

electrode pads. Similar to other forms of tES, TI will be delivered using a low amplitude (4mA or lower) electric current via a minimum of two and a maximum of 24 scalp electrodes. We will use computer simulations to optimize the location of the electrodes to maximize the amplitude of the envelope modulation in the targeted area (calcarine cortex subregions) while minimizing its amplitude in the neighboring or overlying cortex. Electrodes will be fitted with fiducial markers for post-processing localization of the electrodes in MRI. TI stimulation will be applied for up to 10 min (with 5-10s ramping-up and ramping-down periods) with a difference frequency of 1-80Hz.

As compared to modern tES, these values are reduced in duration, frequency and intensity relative to tES guidelines ("up to 60 minutes", frequencies up to 10kHZ), but follow overall guidance for ramping-up and ramping down periods (Antal et al 2017, Clin Neurophysiology).

Control conditions will include non-TI controls (i.e. stimulation at a single frequency equal to the difference frequency of TI stimulation, thus no envelope of stimulation). TI delivered at the carrier frequency only, or TI with alternative difference frequencies expected to be suboptimal. The carrier frequency, as well as the current amplitude, will be selected according to the animal data, scaled according to the computer models, and governed by safety guidelines and considerations.

B. Statistical Considerations

a. ***Sample Size Justification:*** This study aims to assess the feasibility, focality, and steerability of TI stimulation in humans. Pilot testing with 9 healthy participants demonstrated the feasibility and safety of this study, and 9 participants were sufficient to identify a neurophysiological effect upon BOLD signal.

We aim to complete at least 12 subjects, but up to 20 will be entered to allow for attrition.

b. ***Data Analysis:*** This study has a primary aim of assessing visual discrimination thresholds before, during and after TI stimulation. These values will be entered into a repeated measures ANOVA to assess the impact of TI stimulation, with the primary outcome being an interaction between TIME factors (before, during and after TI) and visual field location (stimulated with TI vs unstimulated). All analysis will be conducted in either SPSS or R.

Safety information will be tabulated as frequency of expected events (e.g. headache), and non-conforming safety information will similarly be tabulated and reported.

C. Subject Selection

The population of the Greater Boston area is 84% Caucasian, 9% African-American, 5% Hispanic and 2% Asian, with relatively similar numbers of men and women. Participants of any gender, race, ethnicity, religion, and sexual orientation will be included. All efforts will be made to include equal numbers of both genders and to include minorities in the studies in equal proportions to the Boston area.

Inclusion Criteria:

- Normal healthy volunteer
- 18-45 years of age
- Normal or corrected-to-normal vision
- Right handed

Exclusion Criteria:

- Visual impairment that may interfere with eye tracking equipment (for example glasses with high reflection)
- Any current or past history of a psychiatric disorder
- Any current or past history of neurological disorders or acquired neurological disease (e.g. stroke, traumatic brain injury), including intracranial lesions
- History of head trauma resulting in prolonged loss of consciousness; or a history of >3 grade I concussions
- Current history of poorly controlled headaches including intractable or poorly controlled migraines
- Any systemic illness or unstable medical condition that may cause a medical emergency in case of a provoked seizure (cardiac malformation, cardiac dysrhythmia, asthma, etc.)
- 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 family history of treatment resistant epilepsy with the exception of a single seizure of benign etiology (e.g. febrile seizures) in the judgment of a board-certified neurologist
- Possible pregnancy. All female participants of child bearing age are required to have a pregnancy test
- Any metal in the brain, skull or elsewhere unless approved by the responsible MD
- Any medical devices (i.e. cardiac pacemaker, deep brain stimulator, medication infusion pump, cochlear implant, vagal nerve stimulator) unless otherwise approved by the responsible MD
- Substance abuse or dependence within the past six months
- Pregnancy; all female participants of child bearing age will be required to have a pregnancy test; any participant who is pregnant will not be enrolled in the study.
- Not on any medications with the exception of birth control unless approved by the responsible MD.
- Inability to hold gaze on a central visual stimulus
- Abnormal automated perimetry results at baseline

Data Safety Monitoring Plan

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

Ongoing Assessment:

The application of tES in the form of temporal interference (TI) stimulation has been minimally studied. Prior tES studies have not reported negative effects, including a study applying multi-day stimulation in healthy subjects (Polania, Nitsche, Korman, Batsikadze, & Paulus, 2012; Santarnecchi et al., 2016).

Previous studies using TMS have evoked phosphenes from the single pulse stimulation, from a number of cortical regions. Electrical stimulation often evokes phosphenes, believed to be of retinal origin (Kar and Krekelberg 2012). Repetitive TMS has been used to induce transient scotomas in healthy participants, which overlap with single-pulse evoked phopshenes (Kammer 1999). Despite a history of transient effects from non-invasive brain stimulation resolving, we will conduct the baseline visual field assessment that will be repeated at the end of each stimulation visit as described above.

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 TI or pre/post assessments, will be collected and recorded using a standard adverse event form. Participants will be asked, in an open-ended way, about the presence of any such events on a daily basis. Additionally, a standard questionnaire for tES-related adverse effects will be completed in the period after every TI session. Intensity of each adverse event will be graded as mild, moderate or severe. If an event occurs that is not expected (e.g. is not described in the research protocol or consent form), that indicates a change from baseline in cognition, and/or requires immediate attention, such as a seizure, the PI (or covering investigator) will be informed in real time to assess the event, advise on immediate care of the participant and to determine the necessary reporting steps. Any events that are serious or unexpected in nature, severity or frequency as compared to the risks described in the study plan will be reviewed by the principal investigator or designee (e.g. a co-investigator) to determine the relationship of the event to the study. Reportable events will be submitted to the IRB per determined policies and will be reported to the DSMB as described below.

Adverse Event Communication with Boston University for the MRI protocol

Adverse events related to the MRI imaging protocol at BU will be monitored and collected by study staff at BU. Following the completion of each subject, the staff at BU will share adverse events that were captured by their study team with the BIDMC study team. This information will be included in the summaries provided to the DSMB. A serious or unexpected adverse event that requires reporting to the BU IRB per the BU PI will be reported to the BIDMC study staff in parallel with the BU reporting procedures. These events will be shared with the DSMB in real time as will any local reportable events.

DSMB

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

General Safety Plan

A licensed physician, credentialed at BIDMC will be available by pager during all tACS and TI visits at BIDMC. Furthermore, the person applying tACS and TI 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 TI 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.

B4. POSSIBLE BENEFITS

As with any study focusing on basic research, the subjects will derive no immediate direct benefit. This will be made clear in the informed consent process. This study could advance the translation of TI stimulation methodology to humans and provide new experimental frontiers to map and modulate the brain with a non-invasive alternative to DBS/TMS/tES with low risk, precise steerable focality, and depth selectivity.

B5. POSSIBLE RISKS AND ANALYSIS OF RISK/BENEFIT RATIO

MRI:
The MRI studies will be performed on a 3-Tesla Siemens magnet, which has been FDA approved. 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 prior to referring to BU for the MRI study 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.

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.

Temporal Interferential (TI) brain stimulation:
The proposed experiment represents an early-phase experiment of a novel technique in humans. The purpose of the experiment is to capture potential risks and adverse effects. We can extrapolate from the rather extensive experience gathered in the past several decades using transcranial electric current stimulation (tES) in the forms of transcranial direct current stimulation (tDCS), transcranial alternating current stimulation (tACS) and transcranial random noise stimulation (tRNS) in humans. All these techniques have been shown to have an extremely beneficial side-effect/risk profile. In the

present study, all recommended safety precautions for transcranial current stimulation will be strictly adhered to by the investigators.

This study uses alternating currents which results in less net charge being applied than in tDCS. There is limited reporting of side effects from tES using alternating currents 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.

Based on the experience with transcranial current stimulation, the following side effects (although unlikely) are possible:

- Sensations reported by subjects under the electrode: (These sensations can sometimes continue throughout and for a brief period following completion of the tES but usually resolve shortly after the initiation of tES)
    - Mild tingling (20-70%)
    - Light itching (30-40%)
    - Slight burning (10-22%)
    - Discomfort or mild pain (10-18%)

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

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

- 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%)

- Although it has never been reported in tES, seizures are a theoretical risk.

Visual Discrimination and Periphery Testing:
Subjects may feel frustrated during the visual tasks. They will have breaks between the tasks to reduce the potential for frustration.

Confidentiality:
All data will be stored within secure BIDMC databases. The data will be stored without identifying information, with a code name assigned to the study participant. Data will be transferred with code names between BU and BIDMC via secure email and secure file transfer for MRI portion of the

experiment.

Risk/Benefit Ratio
The above stated risks are weighted with the potential application of non-invasive brain stimulation methods to impact brain function in healthy participants and various patient populations. Preliminary investigations as described here are necessary to validate the technique. We believe the balance of future application of brain stimulation exceeds the documented risks of transcranial electrical stimulation, and the protocol outlines procedures in place to mitigate potential risks of participation.

B6. RECRUITMENT AND CONSENT PROCEDURES

Recruitment
We will recruit subjects by using targeted flyers, internet postings, and databases of prior subjects (IRB approved data repository IRB #2010-P-000169) expressing an interest in future studies. Past study subjects will be approached by e-mail or by phone. Individuals interested in participating in the study will be instructed to contact a study investigator by telephone or e-mail. Individuals who respond to flyers, recruitment e-mails, or phone messages will be contacted by telephone. Study details will be discussed and preliminary screening will be done if the potential subject expresses interest. If the subject continues to express interest and appears to qualify, the first visit will be scheduled for consent and complete screening.

Consent
Initially, subjects will be pre-screened over the phone, thus initiating the consenting process. If they appear to be eligible for the study following the telephone screening, they will be scheduled to come in for the study if they are interested Due to concerns related to COVID-19, the consent process will begin remotely over telephone or secure video. During the consent session, the investigator will begin by discussing the study purpose, requirements, procedures, possible benefits, potential risks and alternatives. If subjects remain interested in participation, they will review the informed consent document and will be allowed to complete reviewing the information in the informed consent form for as much time as they need. The potential subject will have the chance to ask any questions before giving consent. Investigators will emphasize to each potential participant that his or her participation is voluntary and that there is no consequence for refusing participation. Only when they declare that they are comfortable with all aspects, will they be allowed to offer consent. Upon arrival for the first in-person visit, the informed consent will be signed in person by the subject and a member of the study staff. All subjects will be provided with copies of the signed consent form.

Subject Protection
This study uses healthy controls. It is not anticipated that these subjects would be considered vulnerable. To the best of our ability, we will minimize any possibility of coercion or undue influence placed on subjects.

B7. STUDY LOCATION

Privacy

Phone conversations will be limited to providing information about the study and to pre-screening questions. If potential subjects are more comfortable answering questions in person, they will be invited to come in for the screening. At the screening visit, informed consent and details of the study will be discussed in a private room in the Berenson-Allen Center to encourage free exchange of information and only questions pertinent to study participation will be asked.

Physical Setting

Recruitment and screening procedures will take place by study staff at the Beth Israel Deaconess Medical Center. Data will be stored at BIDMC and data analysis will occur at BIDMC or Boston University. In the event that some specialized processing algorithms are run on computers outside BIDMC only de-identified data will be transmitted. MRI scanning will take place at Boston University Cognitive Neuroimaging Center under a separate protocol. All TI stimulation experimental sessions will take place in the Berenson-Allen Center at BIDMC.

B8. DATA SECURITY

All data will be de-identified with the use of unique subject identifiers per HIPAA guidelines. Names will not be provided to external sources, nor will any identifying marker be published in which a participant could be distinguished. 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. De-identified data will be stored on computers protected by individual user passwords to restrict access, using available secure data basing suites of REDCap and XNAT when possible. The XNAT will be hosted and managed by BIDMC and will be kept behind the firewall for security purposes.

MRI scan images will be sent to BIDMC from BU using a secure file transfer. Any paper forms from BU (e.g. adverse event forms) will be scanned and sent to BIDMC via encrypted email. Medical records from outside providers will be requested and obtained via secure email or fax.

Information that will be shared with BU for the MRI study will include PHI that is required to contact the participant for scheduling. All information will be provided via secured email and/or secure file transfer.

Data will be shared with McLean Hospital. As a co-investigator and collaborator at McLean Hospital, Mark Halko, PhD will be on BIDMC premises during study visits to help collect data and will have in-person access to the participants. He will have all data collected by the BIDMC research staff. This data includes PHI.

De-identified data will be sent to the National Institute of Mental Health Data Archive (NDA). All data will be labeled with a Global Unique Identifier (GUID) that is obtained through a system provided by the NDA. Participants will have the option of opting in or out of having their data shared with the NDA.

B9 Multi-Site Studies

Is the BIDMC the coordinating site? ☒ Yes ☐ No

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

Research teams from both locations will communicate via phone, email or conference calls on a monthly basis or more, as needed. In these interactions, teams will assess safety and review study progress.

Adverse events that occur at BU will be communicated to BIDMC study staff for completion of DSMB, and IRB communication as described above in the "Data Safety Monitoring Plan".

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

Data will be shared with a collaborator at Hebrew Senior Life, specifically, Dr. Alvaro Pascual-Leone. De-identified MRI data will be shared with a collaborator at Northeastern University, specifically, Sumientra Rampersand, PhD. Any data that is sent to HSL and Northeastern University will be de-identified and sent via encrypted procedures per BIDMC policy.

B10 Dissemination of Research Results

We will send a follow-up e-mail thanking subjects 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.