# Effect of tACS Stimulation on Alpha Oscillations

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**Co-Investigator:** *N/A*

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| AE | Adverse Event/Adverse Experience |
| ANOVA | Analysis of Variance |
| CFR | Code of Federal Regulations |
| CNS | Central Nervous System |
| Co-I | Co-Investigator |
| CRF | Case Report Form |
| CTRC | Clinical Trials Research Center |
| DMV | Department of Motor Vehicles |
| eCRF | Electronic Case Report Form |
| EEG | Electroencephalogram |
| FDA | Food and Drug Administration |
| GCP | Good Clinical Practice |
| HIPAA | Health Insurance Portability and Accountability Act |
| Hz | Hertz |
| ICF | Informed Consent Form |
| LAR | Legally Authorized Representative |
| NIH | National Institutes of Health |
| NRB | Neurosciences Research Building |
| OHRE | Office of Human Research Ethics |
| OHRP | Office for Human Research Protections |
| PHI | Protected Health Information |
| PI | Principal Investigator |
| SAE | Serious Adverse Event/Serious Adverse Experience |
| tACS | Transcranial Alternating Current Stimulation |
| tDCS | Transcranial Direct Current Stimulation |
| TMS | Transcranial Magnetic Stimulation |
| UE | Unexpected Event |
| UNC | University of North Carolina |
| UNC-CH | University of North Carolina at Chapel Hill |
| US | United States |

## STUDY SUMMARY

| | |
|---|---|
| **Title** | *A pilot, randomized, double-blind, crossover study investigation the effects of 10Hz transcranial alternating current stimulation on the immune response to psychological stress* |
| **Short Title** | *AIMS* |
| **Protocol Number** | *Version 1.1* |
| **Phase** | *Pilot* |
| **Methodology** | *Double-blind, randomized, active sham controlled* |
| **Study Duration** | *This study will take two months to complete.* |
| **Study Center(s)** | *This is a single-site study performed at the University of North Carolina at Chapel Hill.* |
| **Objectives** (Purpose) | *The primary objective of this study is to explore the effects of alpha oscillations on the relationship between the immune system and stress response.* |
| **Number of Subjects** | *20* |
| **Diagnosis and Main Inclusion Criteria** | *Eligible participants will be healthy adults between the ages of 18-35 with no history of autoimmune disease or mental or psychiatric disorder.* |
| **Description of Intervention** (Procedures/methods) | *The participants will be randomized into one of two arms: either 40 minutes of sham tACS or 40 minutes of 10 Hz tACS while in a relaxed, yet experimentally controlled state, by watching a nature movie such as "Reefscape" during stimulation.* |
| **Related IRB Applications** | *N/A* |

# 1 KEY ROLES

## 1.1 INDIVIDUALS

| | |
|---|---|
| **Principal Investigator** | Flavio Frohlich, PhD |
| **Co-Investigator** | *N/A* |
| **Medical Monitor** | *N/A* |

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## 1.2 INSTITUTIONS

The University of North Carolina at Chapel Hill

## 1.3 OPTIONAL

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## 1.4 FUNDING SOURCES

Please list below the funding sources for this project:

| Sponsor Name | UNC Ramses Number | Sponsor Type | Prime Sponsor Name | Prime Sponsor Type | Sponsor/Grant Number |
|---|---|---|---|---|---|
| NIH National Institute of Mental Health (NIMH) | N/A | Federal | N/A | N/A | R01MH101547 |

**External Funding**: This project is externally funded but UNC-CH is not the direct recipient of federal funds.

**UNC-CH Funding**: This project is not funded through UNC-CH.

**Classified**: This project is not classified.

# 2 INTRODUCTION

This document is a protocol for a human research study. This study is to be conducted according to U.S. and international standards of Good Clinical Practice (FDA Title 21 Part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

## 2.1 BACKGROUND

The field of psychoneuroimmunology investigates to the interaction between the brain and the immune system, regulated by psychological and behavioral factors (Kiecolt-Glaser et al, 2002). There are two components of the immune system: innate immunity, which generally responds to threats, and adaptive immunity, which forms specific responses to invaders up to days after the target is recognized (Segerstrom & Miller, 2004). Studies in psychoneuroimmunology have found decreased adaptive immune functioning in individuals subjected to ongoing stress, such as medical students who are administered vaccinations during ongoing periods of high stress (Kiecolt-Glaser et al, 2002). However, lab-induced stressors produce a quick change in markers of innate immunity.

Upon encountering an acute psychological stressor, such as a scary movie or difficult exam, the sympathetic nervous system activates to ready the body for action. Often called the “fight or flight” response, this activation includes heart rate elevation, increased sweat secretion, and vasoconstriction of blood vessels (Segerstrom & Miller, 2004). These effectors are stimulated by norepinephrine, a neurotransmitter that binds to adrenergic receptors on target cells to carry out the sympathetic response. Natural killer (NK) cells – one of the primary components of innate immunity – also have adrenergic receptors; therefore, a sympathetic response to a stressor also stimulates NK activity. Studies involving lab-induced stressors have demonstrated this relationship through a correlation between norepinephrine and NK activity (Schedlowski et al, 1993). Immediately after the stressor, NK levels rise to almost double the baseline value, a peak that is accompanied by a similar rise in norepinephrine levels. Within an hour, NK activity drops to a level significantly below baseline, as the effects of the stressful event and sympathetic activation returns to normal. If this process is repeated, such as during exams or other periods of prolonged acute stress, the fluctuations in NK activity can lead to issues in immune functioning (Kiecolt-Glaser et al, 2002).

## 2.2 INVESTIGATIONAL AGENT

Transcranial Alternating Current Stimulation (tACS) is one method that has been demonstrated to enhance alpha oscillations in healthy participants by applying weak electrical currents to the scalp to modulate rhythmic brain activity patterns (Lustenberger et al, 2015). Alpha oscillations, which occur in frequencies between 8-12Hz, have been correlated with a relaxed emotional state, and EEG recordings of individuals who are meditating show an increase in alpha in the frontal lobe (Lagopoulos et al, 2009; Takahashi et al, 2005). Additionally, this increase in alpha during meditation has been negatively correlated with sympathetic activation, further supporting a relationship between alpha oscillations and decreased stress.

## 2.3 DOSE RATIONALE

10Hz tACS will be applied because this frequency corresponds with the alpha band of 8-12Hz and will therefore increase alpha oscillations. This increase should produce a decreased sympathetic stress response, as indicated by the previously mentioned relationship between alpha and the sympathetic nervous system.

## 2.4 STUDY AIMS/HYPOTHESES

*NULL HYPOTHESIS*. 10Hz tACS does not decrease immune response compared to active sham.

*ALTERNATIVE HYPOTHESIS*. 10Hz tACS decreases immune response compared to active sham.

# 3 SUBJECT SELECTION AND WITHDRAWAL

A total of 20 participants will be recruited for this study and all data will be collected at UNC-CH. No specific plans have been made to enroll participants from vulnerable populations.

## 3.1 INCLUSION CRITERIA

In order to be eligible to participate in this study, a participant must meet all of the following criteria:

- Between the ages of 18 and 35 years
- Capacity to understand all relevant risks and potential benefits of the study (informed consent)
- Willing to comply with all study procedures and be available for the duration of the study
- Speak and understand English

## 3.2 EXCLUSION CRITERIA

A potential participant who meets any of the following criteria will be excluded from participation in the study:

- Diagnosis of eating disorder (current or within the past 6 months)
- Diagnosis of OCD (lifetime)
- ADHD (currently under treatment)
- Neurological disorders and conditions, including, but not limited to:
    - History of epilepsy
    - Seizures (except childhood febrile seizures and ECT-induced seizures)
    - Dementia
    - History of stroke
    - Parkinson's disease
    - Multiple sclerosis
    - Cerebral aneurysm
    - Brain tumors
- Medical or neurological illness or treatment for a medical disorder that could interfere with study participation (e.g., unstable cardiac disease, HIV/AIDS, malignancy, liver or renal impairment)
- Prior brain surgery
- Any brain devices/implants, including cochlear implants and aneurysm clips
- Traumatic brain injury
- (For females) Pregnancy or breast feeding
- Personal or family history of mental/psychiatric disorder (e.g., anxiety, major depressive disorder, schizophrenia, etc.)
- Diagnosis of an autoimmune disorder
- Failure to pass a urinary drug test at the first session.
- Anything that, in the opinion of the investigator, would place the participant at increased risk or preclude the participant's full compliance with or completion of the study

**Justifications for any exclusions based on race, gender, or ethnicity:** Non-English speaking individuals are excluded because the ability to accurately and completely communicate study information, answer questions about the study, and obtain consent are necessary.

**Justification for excluding women or women who become pregnant:** Pregnant participants will be excluded despite the fact that theoretical risk to mother or fetus is exceedingly small, since no safety data for pregnancy is known to exist for tDCS/tACS studies. We will verify pregnancy status via a urine pregnancy test for all female participants prior to receiving treatment on Day 1 of Stimulation.

## 3.3 STRATEGIES FOR RECRUITMENT AND RETENTION

### 3.3.1 RECRUITMENT

The target population of this study includes healthy individuals between the ages of 18 to 35 pulled from UNC Chapel Hill and the surrounding area, including approximately 30,000 candidates for participation. Recruitment will occur via two methods: flyers to be distributed at major locations on the campus (libraries, gyms, classroom buildings, etc.) and a mass email to all individuals with a UNC-affiliated email address.

### 3.3.2 RETENTION

The retention strategy includes a payment schedule of two times per participant, corresponding with the two sessions. The second session will involve a bonus payment for completion of the study. Research staff will send participants a reminder text the day before each session, and will be available by email and phone at all times.

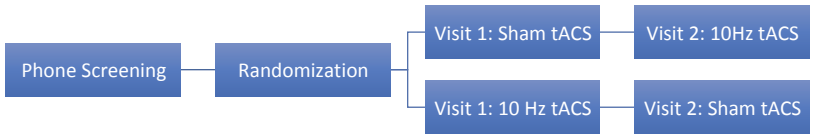
# 4 BASIC STUDY DESIGN

The design for this study is a pilot, randomized, double-blind, sham-controlled, clinical trial which will be used to demonstrate feasibility and collect effectiveness data for further refinement of a tACS approach and for the subsequent design of a follow-up, multi-site, large scale study. We are seeking 20 healthy participants, both male and female, between the ages of 18-65. All women of child-bearing potential will be asked to take a pregnancy test during the initial session in order to determine eligibility for the study.

This is a double-blind, randomized crossover study. We estimate 2 months to complete study enrollment.

Participants will be randomly assigned to one of 2 arms.

**Figure 1.**




Active sham treatment will include 10 seconds of ramp-in to 1 minute of 10 Hz tACS with a ramp-out of 10 seconds for a total of 80 seconds of stimulation. The choice of an active sham is motivated to enhance success of patient blinding by mimicking skin sensations associated with tACS. 10 Hz tACS will have a 10 second ramp-in and ramp-out with 40 minutes of stimulation for a total of 2420 seconds of stimulation. Stimulation waveform is a sine-wave with peak-to-peak amplitude of 2 mA. In both arms, participants will stay in a relaxed yet controlled state by watching a nature movie (“Reefscape”) during stimulation.

Eligible participants will have a total of 2 visits, each lasting approximately 3 hours and separated by at least 1 week. Total participation will include about 6 hours over the course of two weeks. All time estimates take into consideration breaks and time variance in administration.

## 4.1 TREATMENT ASSIGNMENT PROCEDURES

Participants will be randomized into one of 2 arms (*see Figure 1 above*). This is a double-blind study, so neither the participant nor the research will know which treatment the participant is receiving, if any.

### 4.1.1 RANDOMIZATION PROCEDURES

Charles Zhou will randomize 40 6-digit stimulation codes which will be used by the study coordinator and research assistants and will be linked to the participant numbers of enrolled participants. These stimulation codes are directly linked to which treatment participants receive (sham or 10 Hz tACS) at each session, and will be used with the XSCITE 100 stimulator. An unblinded code sheet that matches these stimulation codes to treatment arm will be kept by Charles Zhou and will not be available to the study coordinator or research assistants.

The unblinded code sheet will have the following information:

1. The initial identifier codes for all potential participants
2. Stimulation code: 6-digit numerical code for the stimulation session
3. Condition number: Numerical code for the condition
4. Condition name: Name of the condition

A copy of this code sheet with condition number and condition name REMOVED is provided to the study coordinator and research assistants. This blinded code sheet will be used to ensure the correct stimulation code is provided for each session.

These linked codes ensure that the study coordinator and research assistants are kept blinded to which treatment each participant receives. Please see *Data and Safety Monitoring* for more information on unblinding this information.

# 5 STUDY SCHEDULE

It is important to note that consent, scales, and experiments will all take place in a private room. Any phone calls will take place in a private lab environment as well.

## 5.1 SCREENING

The screening process will involve a telephone call with the potential participant to inform of expectations and determine eligibility. A researcher will conduct a screening with each participant prior to scheduling the first session. If a participant is determined to be ineligible, data from the screening will be retained.

## 5.2 STIMULATION SESSIONS

Participants will undergo two sessions with the same procedure; the only change will be the stimulation condition.

### 5.4.1 SESSION 1

In the initial session, participants will be explained the procedure and reminded of their expectations and rights (informed consent). After obtaining consent, a urine drug test and pregnancy screening will be administered. Once they are both determined to be negative, the stimulation, EEG, and heart rate electrodes will be applied. Participants will then undergo a ten-minute resting state block to record EEG activity. Participants then provide a baseline saliva sample and receive 40 minutes of either 10Hz tACS or sham stimulation (depending on the randomized condition) while watching the 'Reefscape' video. After the stimulation, the participant will complete the stimulation adverse effects questionnaire, and will then provide a final saliva sample. The study coordinator will remove all equipment, and the participant will fill out the final two questionnaires and receive $20 in compensation.

### 5.4.2 SESSION 2

The procedure for the second session will follow the same timeline as the first, with the exclusion of informed consent, urine drug test, and pregnancy screening processes. Participants will receive $40 in compensation at the end of this session.

### 5.4.3 UNBLINDING PROCEDURES

There are no current plans to systematically unblind participants to the treatment they may or may not have received during the clinical trial. However, following the completion of data collection, participants may contact the Frohlich Lab for unblinding information.

# 6 STUDY PROCEDURES/EVALUATIONS

## 6.1 SELF-REPORT MEASURES

During the telephone screening, researchers will collect demographics, which include medical history and medication history. In addition, several other self-report measures will be used throughout this study. These measures are listed below and can be found in the attached documents.

A. THE PERCEIVED STRESS SCALE (PSS) is a 10-item self-report assessment using a 5-level Likert scale to assess the degree to which an individual perceives life events as stressful in the past month. This scale is the most commonly used measure of self-reported stress, and produces results that are valid for 4-8 weeks after administration (Cohen & Williamson, 1988).
B. THE STATE-TRAIT ANXIETY INVENTORY (STAI) is a 20-item self-report assessment that assesses either temporary or chronic anxiety. For the purposes of this study, the state version will be used to measure anxiety as a result of the stress condition. The STAI is commonly used to assess both types of anxiety, and has applications in both clinical and research settings (Spielberger et al, 1983).
C. THE BEHAVIORAL INHIBITION AND BEHAVIORAL APPROACH SYSTEM SCALES (BIS/BAS) are a set of 24 questions used to assess an individual's sensitivity to approach vs. inhibition in motivating behavior. This scale is commonly used to measure behavior and has been demonstrated to be reliable (Carver & White, 1994).
D. THE CONNOR-DAVIDSON RESILIENCE SCALE (CD-RISC) is a 25-item self-report assessment using a 5-level Likert scale to assess the degree of resilience an individual displays to stressful events. This scale has been shown to be a reliable measure of resilience, correlating with improvement during treatments designed to improve anxiety disorders (Connor & Davidson, 2003).
E. THE POSITIVE AND NEGATIVE AFFECT SCHEDULE (PANAS) is a 20-item self-report assessment using a 5-level Likert scale to assess the positive and negative affect of an individual over the past week. This scale has been shown to be a reliable measure of both positive and negative affect with little subjectivity to demographic variables (Watson et al, 1988).

## 6.2 SPECIAL ASSAYS OR PROCEDURES

Each participant will receive two days of stimulation, separated by a week. One session will involve 10Hz tACS, and the other will involve active sham; the order of stimulation condition will be randomized for each participant. For more information on the stimulation procedures, see section *7.2 Preparation and Administration of Study Investigational Product*.

## 6.3 SAFETY MEASURES

We will be monitoring the safety of our participants throughout the study with the following measures. These measures are listed below and can be found in the attached documents.

A. A stimulation adverse effects questionnaire used in previous studies (IRB #14-1622, #14-3285, and #14-0600) will be administered at the end of each stimulation session. This questionnaire will be used as a safety measure and to collect data on participant experience. Please see *10.1 Safety Parameters* for more information.

## 6.4 LABORATORY EVALUATIONS

### 6.4.1 SCREENING LABORATORY EVALUATIONS

A urine pregnancy test will be performed for any female participant who is unable to confirm pregnancy status. All participants will also undergo a urine drug test.

INSTRUCTIONS FOR SPECIMEN PREPARATION, HANDLING, AND STORAGE. For this laboratory evaluation, results are available after only a few minutes. Once the results are clear, the researcher will make a note and the sample will be disposed. All samples will be handled using single-use disposable medical gloves.

### 6.4.2 SALIVA SAMPLES

We will be collecting a saliva sample at the first session. This sample will be used to test for a single nucleotide polymorphism in the BDNF gene whose presence may have an influence on effectiveness of brain stimulation. Within the central nervous system, BDNF regulates survival, proliferation, and synaptic growth as well as directly influences synaptic plasticity in the adult human brain (Antal et al., 2010). Egan et al. (2003) demonstrated that Val66Met, a single nucleotide in the BDNF gene, has function consequences in healthy humans, including decreased episodic memory and hippocampal inducing a reduction in recall capacity. This polymorphism is common in over one third of the Caucasian population (65% Val66Val to 35% Val66MET) (Pezawas et al, 2004; Hariri and Weinberger, 2003). Kleim et al. (2006) found that individuals with the Val/Val polymorphism respond to tDCS and transcranial magnetic stimulation (TMS) treatments with expected change, whereas, individuals expressing Val/MET allele do not. These authors indicate the difference to be caused by the impairment in synaptic plasticity caused by the Val/MET allele. These findings suggest that individual efficacy of treatments using brain stimulation may be partially genetically predetermined and should be taken into account when preforming such procedures. Accordingly, we will conduct genotyping of all participants in this study in order to assess BDNF status. We will perform exploratory analyses in which we group participants by BDNF status.

We will also be collecting two saliva samples at each session to test for a cytokine panel, salivary alpha-amylase, and cortisol as measures of immunity and stress.

INSTRUCTIONS FOR SPECIMEN PREPARATION, HANDLING, AND STORAGE. The saliva samples for BDNF testing will be collected using 2mL DNA collection kit from DNA Genotek. Before sample collection, it is imperative that the participant does not eat, drink, smoke or chew gum for at least 30 minutes before providing a sample. Once the participant provides the 2mL sample, the collection tube is closed and a liquid from the lid will be released into the tube. The original lid will be removed and replaced with a small cap and the tube will be agitated for 5 seconds. The sample is then returned to the plastic packaging and labeled with the date of collection, the study name, and the participant ID. These samples are kept in a secure location until the completion of data collection.

Saliva samples at each session will be collected using the Passive Drool collection kit from Salimetrics. These samples will be labeled with the study name and participant ID, and frozen immediately after collection until completion of all data collection.

# 7 STUDY INVESTIGATIONAL PRODUCT

## 7.1 DEVICE DESCRIPTION

We will be using the XCSITE100 stimulator designed in the Frohlich Lab for investigational purposes. The device is not implanted and has not been designed for or being used to support or sustain human life. This device does not have a potential for serious risk to the health, safety, or welfare of the participant. There has never been an instance of serious side-effect reported due to the use of transcranial brain stimulation. Previous studies in the Frohlich lab that used comparable devices (i.e., the commercial, CE-certified Neuroconn Plus stimulator) have always been classified as "non-significant risk" by the full UNC IRB. The Neuroconn Plus stimulator and the XCSITE100 stimulator are electrically equivalent and provide the same stimulation.

The XSCITE100 is the first non-invasive brain stimulator designed for research purposes to provide an active sham for tACS and record the stimulation output for later validation. This stimulator may apply either tDCS or tACS for up to 40 minutes (2400 seconds) with appropriate current ramp-up at the beginning of stimulation and ramp-down at the end of stimulation. Both tDCS and tACS may be applied for currents between 100 µA and 2 mA (peak-to-peak for tACS).

The stimulator has two main components:

1. Android tablet with user interface application (i.e., App)
2. Stimulator with:
    a. Microprocessor
    b. Function generator chip
    c. Voltage controlled current source
    d. Safety circuitry

To ensure appropriate blinding for each stimulation session, there are designated unblinded individuals to ensure the appropriate stimulation parameters are applied to each participant. These individuals will not interact with participants and will only be involved with the creation of a study file and validation of stimulation waveform.

## 7.2 SAFETY FEATURES

*Current Sensor Circuit*. A 33.2 Ω sense resistor is placed in series with the stimulation electrodes on the high side. Since high-side current sensing is used, any short circuit of the electrode terminals to ground will be detected. The stimulation current flows through this resistor and creates a voltage. The voltage across this resistor is sensed and amplified by the AD628 difference amplifier. The gain of the difference amplifier is set to 9.9039. The current sensor voltage is then shifted before it is read by the microprocessor and the hardware current safety feature.

*Voltage Sensor Circuit.* The differential voltage across the electrodes is measured so that the impedance can be calculated. The voltage is measured by buffering the positive electrode and negative electrode each with a unity gain op-amp circuit. The voltage output is then shifted before it is read by the microprocessor using the same level shifting circuit described in the current sensor section.

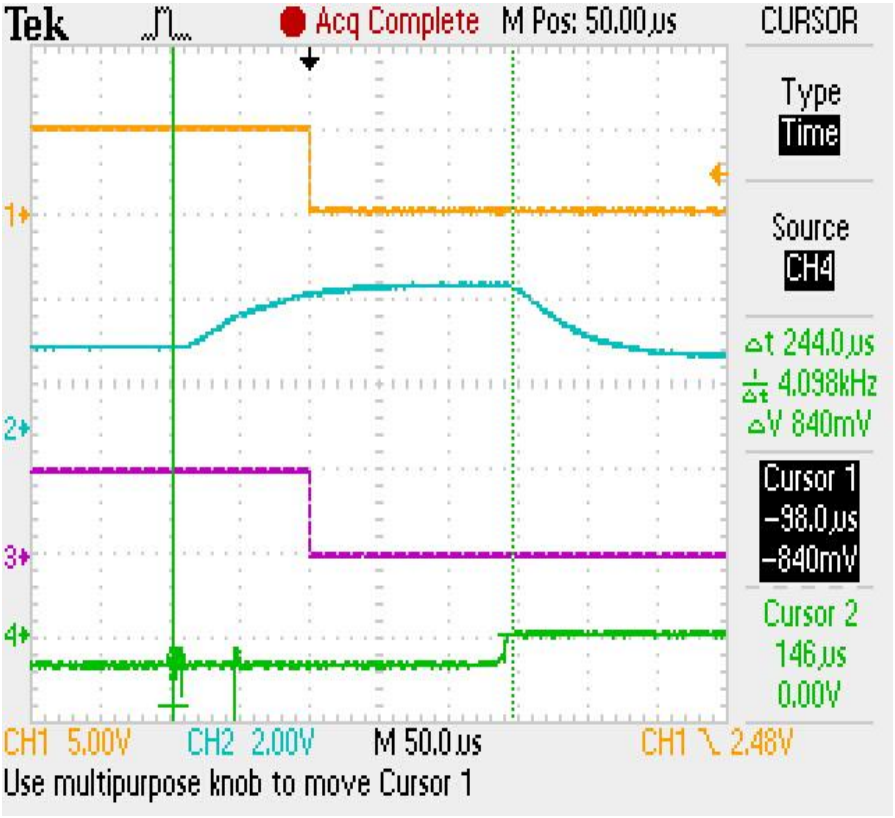
The device is equipped with 4 different stages of safety precautions, all of which protect the participant from high currents. The stages are as follows:

1. AUTOMATIC SOFTWARE CURRENT CUTOFF. The output of the current sensor described above is read by a microprocessor, which compares the reading to a value of ±3 mA peak. If the current exceeds these limits,

stimulation is stopped, a relay in series with the electrode is opened, and the power supply used for stimulation is turned off. The user is then given the option to investigate the issue , and cancel or resume the test. Since high-side current sensing is used (described above), any short circuit of the electrode terminals to ground will be detected.

2. AUTOMATIC HARDWARE CURRENT CUTOFF. The output of the current sensor is fed into a pair of comparators which detect if the current exceeds ±4.5 mA. If so, the fault is latched such that the relay in series with the electrodes is opened. Additionally, the microprocessor is notified of this instance through an interrupt. Upon this interrupt, the microprocessor immediately stops stimulation and the power supply used for stimulation is turned off.

Figure 1. Example of a successful hardware cutoff function




Yellow: Relay control line

Green: Current through electrode (goes from negative current to zero)

Blue: Current sensor output (has lowpass filter)

3. PERMANENT HARDWARE CURRENT CUTOFF. A 5 mA fast-acting fuse is in series with the electrode connector. If the above two over-current detection methods fail, the fuse will below, and the stimulator will no longer be electronically connected to the device.
4. POWER SUPPLY FUSE. Finally, if for no other reason the entire device draws too much current, the main power supply fuse is blown. This fuse is sized with a cutoff of 200% of steady-state operating current.

## 7.3 PREPARATION AND ADMINISTRATION OF STUDY INVESTIGATIONAL PRODUCT

After participants have completed the questionnaires, they will be comfortably seated. The research team will first measure their heads electrode placement using the 10-20 system. Participants will then be fitted with the 3 electrodes for stimulation. The participant will be in the relaxed yet, experimentally controlled state by watching a nature movie. One session of stimulation will be performed per visit for 40 minutes. During the 10 Hz tACS

condition, participants will have a 10 second ramp in and ramp out with 40 minutes of stimulation for a total of 2420 seconds. Stimulation waveforms are sine-waves with a peak-to-peak amplitude of 2 mA. The sham stimulation will include 10 seconds of ramp in to 1 minute of 10 Hz tACS with a ramp out of 10 seconds for a total of 100 seconds of stimulation. Electrodes will be saline soaked, 5x5cm and placed over F3 and F4 with a 5x7cm placed over CZ as a return electrode.

Stimulation devices will be preprogrammed and codes will be randomized to one of the two experimental arms. Researchers will enter the participant-specific code into the App that controls the stimulation and monitor participants during the 40 minutes of the stimulation.

The study coordinator and/or the research assistant will be thoroughly trained and have trainings documented on the transcranial stimulation device and will be present during all stimulation sessions. *Please see Appendix F for an example of the training documentation log.* To monitor side effects of stimulation a questionnaire will be administered after each stimulation session. *Please see Attachment 1 for an example of the stimulation questionnaire.*

## 7.4 ASSESSMENT OF PARTICIPANT COMPLIANCE WITH STUDY INVESTIGATIONAL PRODUCT

Compliance for this study includes attending both sessions. Individuals who miss a session and are unable to reschedule within one week will be dropped from the study.

# 8 POTENTIAL RISKS AND BENEFITS

## 8.1 BENEFITS TO SUBJECTS AND SOCIETY

The results of this study have major implications for the use of tACS as a treatment method for alleviating stress and symptoms of stress. One such symptom is compromised immune functioning, which has been demonstrated as detrimental especially in students and other individuals experiencing high levels of acute stress. The results of this study may also prompt further investigation into the relationship between cortical oscillations and immune functioning.

This study is not designed to benefit the individual participants. However, participants may experience some acute or even chronic stress relief as a result of the treatment. There are no serious risks to the participant from the treatment used in this study.

## 8.2 POTENTIAL RISKS

### 8.2.1 PSYCHOLOGICAL

**Emotional Distress**: As part of data collection, participants will undergo a timed mental arithmetic task designed to induce psychological stress. This task is meant to induce a short-term stress response via enhanced secretion of catecholamines. Such a response occurs naturally in the body and has not shown any long-term negative side effects. In order to protect the integrity of results, participants will not be made aware of the purpose of the task until after completion of the final session.

### 8.2.2 PHYSICAL

**Risk of Injury and Discomfort**: Transcranial current stimulation has been used without any reports of serious side-effects for more than a decade. This stimulation made has NOTHING to do with electroconvulsive therapy that applies many orders of magnitude higher stimulation current. Rather, transcranial current stimulation is so weak that it does not cause super-threshold activation of neurons (Frohlich and McCormick, 2010). In particular, tACS has been used without reports of any serious side-effects. Some participants report a transient mild tingling, burning, or itching underneath the electrodes and headache, but no other side effects have been noted. Importantly, it remains unclear if these mild side-effects were caused by the transcranial brain stimulation. In order to monitor these side-effects, we will be administering an adverse effects stimulation questionnaire after each stimulation session to determine whether these effects were experienced. Research personnel present during these sessions will also check in with the participant periodically during the stimulation to see whether they are comfortable. If any side-effect occurs (rated by the participant as stronger than “moderate”) or the participant is experiencing severe discomfort, the stimulation will be immediately stopped.

## 8.3 REFERRALS FOR MEDICAL FOLLOW-UP OR PSYCHOLOGICAL COUNSELING

There is a purely theoretical likelihood that stimulation of neuronal circuits can lead to epileptic discharge. To minimize this occurrence, we screen and exclude patients with personal and family history of neurological conditions from the study. If abnormalities or a seizure is witnessed during the course of the study, a referral will be made to the UNC Department of Neurology for follow-up. In the theoretical event that a seizure is witnessed

that involves the loss of consciousness, the patient will be told not to operate a motor vehicle until cleared by the DMV.

To ensure participant comfort, a study coordinator or research assistant will periodically check in with the participant about any side-effects he/she may be experiencing during each stimulation session. Following the conclusion of the stimulation session, the participant will receive an Adverse Effects Questionnaire to report on any of the side-effects he/she may have experienced. This questionnaire reports side-effects on a likert scale (1=Absent, 2=Mild, 3=Moderate, 4=Severe). If the participant reports side-effects of Moderate to Severe intensity, a study coordinator or research assistant will discuss the side-effects experienced and note this response. The PI will be contacted based on the reported intensity on the Adverse Events Questionnaire and the participant's verbal confirmation of intensity.

### 8.3.1 PREGNANCY FOLLOW-UP

Every female participant will take a pregnancy test on Day 1 of Stimulation. If, after testing negative at Day 1 of Stimulation (meeting inclusion criteria), a participant reports becoming pregnant during the course of the study, she will be withdrawn from further participation. There are no plans to follow participants who become pregnant while enrolled in the study.

# 9 DATA AND SAFETY MONITORING

## 9.1 FROHLICH LAB MONITORING PLAN

The purpose of this monitoring plan is to present the Frohlich Lab's approach to monitoring clinical trials. The plan facilitates compliance with good clinical practice (GCP):

a. The rights and well-being of human subjects are protected.
b. The reported trial data are accurate, complete, and verifiable from source documents
c. The conduct of the trial is in compliance with the currently approved protocol/amendment(s) with GCP, and with applicable regulatory requirement(s)

This section identifies key monitoring activities and specifies the data to be reviewed over the course of a clinical trial. This is a single site, investigator-initiated, clinical trial, so there will be no site monitoring plan in place.

The latest version of the approved IRB application for this clinical trial will be followed at all times. This responsibility falls into the hands of the study coordinator and research assistants. If at any time there is a deviation from protocol, the deviation from protocol log will be filled out. All team members will be trained on how and when to use this log. The most up-to-date IRB application will be on file in the Clinical Trials office in Room 233 of the Medical School Wing C. Deviations will be sent to the IRB every 4-6 weeks (if necessary).

Periodically, study staff should review 3 randomly selected inform consent forms to ensure that (1) these forms have been filled out appropriately, and (2) the consent form process was followed and properly documented. Should any consent form be in violation, the research team will perform and document a complete review of all consent forms.

AE and SAE are clearly defined in this document. Documents of AE and SAE can be found in the study binder on file in the Clinical Trials office in Room 233 of the Medical School Wing C. It is the responsibility of the study coordinator to report all events to the PI in a timely manner (see *9.3 Reporting Procedures*). All AEs and SAEs will be discussed with the PI. For our practices, we have adapted the decision tree provided by the UNC-CH IRB to assist with reporting of such events.

## 9.2 SAFETY OVERSIGHT

Safety oversight will be under the direction of the PI, who will review AEs in real time and make decisions regarding a participant's continuation of the clinical trial.

## 9.3 EARLY WITHDRAWAL OF PARTICIPANTS

### 9.3.1 REASONS FOR WITHDRAWAL

A study participant will be discontinued from further participation if:

- The participant fails to adhere to rules, including stress, exercise, and sleep restrictions
- The participant meets any exclusion criteria (either newly developed or not previously recognized).
- Anything that, in the opinion of the investigator, would place the participant at increased risk or preclude the participant's full compliance with or completion of the study.

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

### 9.3.2 DATA COLLECTION AND FOLLOW-UP FOR WITHDRAWN PARTICIPANTS

We will collect safety data on any participant discontinued because of an AE or SAE. In any case, every effort will be made to undertake protocol-specific follow-up procedures. If an AE has been reported, researchers will help the participant seek the medical care they need and a follow-up will be performed by the PI. In the case of an early withdrawal, the researcher will make a note to file indicating this.

## 9.4 TERMINATION OF STUDY

If a seizure occurs at the time of a study visit, a temporary hold will be placed over the study and further investigation will ensue. This could lead to stopping the study prematurely or continuing on with further safety measures in place. If two seizures are witnessed during the study visits, the entire study will be stopped prematurely. These individuals would be referred for further medical attention. It is very unlikely that a seizure will occur, given that previous studies using tDCS in patients with depression and schizophrenia have had no seizures occur (Berlim et al., 2013, Brunelin et al., 2012).

The study will also be stopped (at least temporarily) if studies provide evidence that transcranial current stimulation caused brain damage or other harmful effects on subjects, either short-term or long-term. Examples of findings that might trigger a safety review are the number of SAEs overall, the number of occurrences of a particular type of SAE, severe AEs/reactions, or increased frequency of events.

The reasons for stopping the study and asking for further investigation include:

- If a seizure occurs during a study visit, a temporary hold will be placed on the clinical trial

The IRB will also be informed promptly and provided the reason(s) for the termination of suspension of by the investigator, as specified by the applicable regulatory requirement(s).

# 10 SAFETY & REPORTING

It is important to assess safety over the course of this study. This section describes in detail how safety is assessed, reporting of Adverse Events, Serious Adverse Events, and Unanticipated Problems. This section is a reference for internal use.

## 10.1 SAFETY PARAMETERS

**STIMULATION SIDE EFFECTS.** A stimulation adverse effects questionnaire used in previous studies will be administered at the end of each stimulation session. This questionnaire will be used as a safety measure and to collect data on participant experience. The adverse effects questionnaire asks participants to respond on a 4 point Likert scale on the severity of symptoms experienced during the stimulation session (1 = absent, 2 = mild, 3 = moderate, 4 = severe). The side effects listed are headache, neck pain, scalp pain, tingling, itching, ringing/buzzing noise, burning sensation, local redness, sleepiness, trouble concentrating, improved mood, worsening of mood, dizziness, flickering lights, and other (specify). Participants are also asked to rate on a 5 point Likert scale how related they believe the side effects to be to stimulation (1 = no relation, 2 = remote, 3 = possible, 4 = probable, 5 = definite).

In addition to this survey, the study coordinator or research assistant will periodically check in with the participant during the stimulation session to assess side effects.

## 10.2 METHODS AND TIMING FOR ASSESSING, RECORDING, AND ANALYZING SAFETY PARAMETERS

### 10.2.1 ADVERSE EVENTS

All AEs, including local and systemic reactions not meeting the criteria for "serious adverse events", will be captured on the appropriate CRF. In addition, the AE Report Form will be completed by the study coordinator (Appendix B). The AE Report Form includes the following:

- What is known about the therapy
- What is known about previous reported side effects
- If the AE occurred in temporal relation to the therapy
- Whether or not the AE improves or disappears when treatment is stopped
- Whether the AE is worsening of baseline symptoms
- Whether the AE is related to concurrent medical condition or medication use

Once complete, this form will be given to the PI and Co-I, who will review, comment, and sign this form. Completed forms will be placed in the participant's folder.

In addition, the study coordinator will document any AE occurrence on the AE log (*Appendix D*), which includes information such as the date of the AE, severity, relationship to the treatment (assessed by the PI and Co-I*), actions taken, and outcome(s). The log will be reviewed and initialed by the PI 72 hours after being completed. All AEs occurring during the clinical trial will be documented appropriately regardless of relationship to tACS. All AEs will be followed to adequate resolution and will be graded for severity and relationship to study treatment. Any medical condition noted at the initial session will be considered at baseline and not reported as an AE.

All AEs will be graded for severity using the following guidelines:

- **ASYMPTOMATIC**. The participant is exhibiting no symptoms due to this event; no treatment needed.
- **MILD**. Event results in mild or transient discomfort, not requiring intervention or treatment; does not limit or interfere with daily activities (e.g., insomnia, mild headache)
- **MODERATE**. Event is sufficiently discomforting so as to limit or interfere with daily activities; may require interventional treatment (e.g., fever requiring antipyretic medication). In the case of a moderate AE, the medical advisor may recommend an over the counter medication.
- **SEVERE AND UNDESIRABLE**. Event results in significant symptoms that prevent normal daily activities; may require hospitalization or invasive intervention (e.g., anemia resulting in blood transfusion).

Changes in the severity of an AE will be documented with the Note to File document (Appendix E) and will be filed in the participant's folder.

***Relationship to Study Products**: The PI will determine whether an AE is associated with the study treatment. The event will be labeled associated if the event is temporally related to the administration of a therapy and no other factors can explain the event. The event will be labeled as not associated if the event is temporally independent of the study treatment and can be explained by external factors, such as major life events.

### 10.2.1 SERIOUS ADVERSE EVENTS

Serious Adverse Event (SAE): An SAE, as defined by the NIH, consists of adverse events that result in death, require either inpatient hospitalization or the prolongation of hospitalization, are life-threatening, result in a persistent or significant disability/incapacity or result in congenital anomaly/birth defect. Other important medical events, based upon appropriate medical judgment, may also be considered Serious Adverse Events if a trial participant's health is at risk and intervention is required to prevent an outcome mentioned.

All SAEs will be recorded on the Serious Adverse Events Form (Appendix B), documented in the UE/SAE log and reported to the IRB. The SAE Form will be completed by the study coordinator, and includes information relating to the onset and nature of the SAE, relationship to the study treatment, seriousness of the SAE, treatment required as a response to the SAE, and outcome. This form will be filed in the participant's folder at the resolution of the event. The study coordinator will complete the UE/SAE log (Appendix D) which includes information such as the date of the event, time at which the study team was informed of the event, details, when the IRB was notified, and the date that the SAE form was completed.

### 10.2.2 UNANTICIPATED PROBLEMS

Unexpected Events (UE) will be recorded on the UE/SAE log (Appendix D) and will include information such as the date of the event, when the study team was informed of this event, details of the event, when the IRB was notified, and whether the SAE form was completed. The IRB will be notified of each UE that may occur during the study.

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to subjects or others to include, in general, any incident, experience, or outcome that meets all of the following criteria:

- unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
- related or possibly related to participation in the research (in the guidance document, possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

If an UE occurs the IRB will be notified and the study will be adjusted as needed to protect the health and safety of the participants. Depending on the nature of the UE, the research protocol, inclusion/exclusion criteria, and informed consent will be changed to reflect the possibility of this event reoccurring. During this time, no new participants will be recruited and the research procedures for currently enrolled participants will be stopped. Each UE will be recorded and reported throughout the study.

## 10.3 REPORTING PROCEDURES

We will be adopting the following table for reporting procedures:

| What Event is Reported | When is Event Reported | By Whom the Event is Reported | To Whom the Event is Reported |
|---|---|---|---|
| Fatal or life-threatening unexpected, suspected serious adverse reactions | Within **24 hours** of initial receipt of information | Investigator | Local/internal IRBs |
| Non-fatal, non-life threatening unexpected, suspected serious adverse reactions | Within **48 hours** of initial receipt of information | Study Coordinator | Local/internal IRBs/Institutional Officials, DSMB |
| Unanticipated adverse device effects | Within **10 working days** of investigator first learning of effect | Investigator | Local/internal IRBs |
| Unanticipated problem that is not an SAE | Within **7 days** of the investigator becoming aware of the problem | Investigator | Local/internal IRBs/Institutional officials |
| All Unanticipated Problems | Within **30 days** of the IRB's receipt of the report of the UP from the investigator | IRB | OHRP |
| | | Investigator | External IRBs |

### 10.3.1 REPORTING OF PREGNANCY

Pregnancy tests will be administered on Day 1 of Stimulation to all women of child-bearing potential. There are no studies that suggest tACS would interfere with pregnancy. However, should a participant become pregnant during the study, their participation will be immediately terminated.

## 10.4 TYPES AND DURATION OF FOLLOW-UP OF PARTICIPANTS AFTER ADVERSE EVENTS

The study coordinator will follow up within one week of an adverse event.

# 11 STATISTICAL PLAN

The statistician for this study is Dr. Flavio Frohlich.

## 11.1 STATISTICAL ANALYSIS STRATEGIES

### 11.1.1 MEASURES OF INTEREST

The primary outcomes of this study will be the quantified changes in cytokine, alpha-amylase, and cortisol activity.

EEG changes will be quantified and analyzed as secondary outcomes.

### 11.1.2 AIMS

AIM 1: Characterize and compare the sham and tACS stimulation conditions in terms of the measures of interest, stated above. These outcomes will be used to decide whether the tACS regimen should be evaluated in a larger controlled trial.

AIM 2: Obtain statistical estimates needed to plan a future, large-scale clinical trial.

### 11.1.2 ANALYSIS

Statistical analyses will be performed to test the null hypothesis indicated in section *2.4 Study Aims/Hypothesis*. Data from the saliva samples will be compared within and between the two treatment sessions for each participant. Within sessions, the post-treatment sample will be compared to both the post-stress and the baseline, to determine the changes in biomarker levels as a result of the stimulation. Then, these two differences will be compared to the same differences from the corresponding treatment session for the participant, in order to determine if the tACS treatment had a significant effect on NK cell count. Biomarkers will also be correlated with each other to confirm the previously established relationship between sympathetic activation and immune response.

A significant difference (p-value less than or equal to 0.05) between the treatment conditions in within-session activity of any of the biomarkers will constitute a rejection of the null hypothesis. Any outcome with a p value > 0.05 will be regarded as inconclusive. Confidence intervals of 95% will be reported for all statistical estimates. Additionally, interpretation of outcomes will focus on estimated changes in levels of biomarkers, rather than just significance. Data will be represented in a mixed linear model to account for correlated data and unequal variances. In the event that the primary outcomes do not produce significant results, exploratory analyses will be conducted on the existing data to determine new hypotheses.

Any incomplete or missing data will be excluded from analysis; if time permits, further data collection will be conducted to account for the loss.

## 11.2 SAMPLE SIZE DETERMINATION

This clinical trial represents a pilot study. A pilot study is a clinical trial that is conducted to decide whether a new treatment should be tested in a large controlled trial; therefore, we do not calculate sample size. This study can be

considered a pilot study, as it is the first time this specific treatment will be performed on this clinical population. The results from this study will be used to determine sample sizes for future, large-scale clinical trials.

In addition, we have to restrict the number of included participants to a small sample size due to limited funding resources.

# 12 DATA HANDLING AND RECORD KEEPING

The study coordinator and research assistants are responsible for the accuracy, completeness, legibility, and timeliness of the data reported.

## 12.1 PHI AND HIPAA

Information about study participants will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from the participants in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a participant revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of participant authorization. For participants that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e., that the subject is alive) at the end of their scheduled study period.

## 12.2 CONFIDENTIALITY

Data will be stored in a password-protected cloud-based data system that does not contain any patient information. Individual records are referred to by dummy identifiers that cannot be traced back to the study participants except with the master code list that is stored separately in a secured location. Source documents (i.e. paper forms) will be kept in a locked file cabinet in a locked office. The key linking dummy identifiers with participant information will be securely located separate from all other data collected, and will never appear in an electronic format.

### 12.2.1 ACCESS TO SOURCE DOCUMENTS

The research coordinator, research assistants, and PI will have access to all of the source documents collected over the course of the study.

Data will stay on a password-protected computer. Subsequently, a copy will be processed on a separate, password-protected desktop computer in the Frohlich Lab (Neuroscience Research Building 4109).

### 12.2.2 SENSITIVE INFORMATION

Sensitive information may be collected during the screening process in order to determine eligibility (medical history, recreational drug use, etc.). Such information will be de-identified and stored in a secure, locked file cabinet, and will not be used in any further proceedings in the study.

### 12.2.3 OTHER

Please note that there is no significant risk of deductive disclosure in this study. In addition, none of the groupings or subgroupings used in analysis will be small enough to allow individuals to be identified.

## 12.3 SOURCE DOCUMENTS

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Source data include:

**PARTNERS HUMAN RESEARCH COMMITTEE (IRB).**

- All IRB correspondences are documented
- The study staff is IRB approved prior to performing any study procedures
- Adverse events and deviations are reported to the IRB per current guidelines and stored appropriately
- All versions of the IRB protocols and informed consent forms are on file

**INFORMED CONSENT.**

- Ensure that participant identification is not recorded on the ICF (i.e., no participant ID)
- There is documentation that the participant is given a copy of the consent form
- The participant and study representative signed and dated the consent form for him/herself
- The participant initialed and dated all appropriate pages on the informed consent form
- Note to file (Appendix F) made for any informed consent deviations
- Ensure a valid (current version date) copy of the consent form was used

**PROTOCOL DEVIATIONS.**

- Any and all protocol deviations (exceptions and violations) are documented in the participant folder and reported to the IRB as required

**OTHER SOURCE DOCUMENTS.**

- Each participant folder will contain a checklist to ensure that all source documents are administered and collected properly. The checklist will be dated by the researcher for each time an assessment is administered
- Review participant folders to ensure the accuracy, completeness, and legibility of the data.
- Any correction made to the source documents is dated, initialed, and explained. The original entry should not be obscured.
- The protocol-specific source documents are on file.
- Source documents are completed in ink.
- Note to files (Appendix F) are made for missing or incomplete data and to explain any discrepancies or additional comments.

**DNA.**

- Participant names will not be on any of the samples collected. DNA is sequenced to check for one nucleotide. When testing is performed, only de-identified information is shared with an outside party. This information will not be shared with anyone outside of the study personnel, including the participant.

## 12.4 DATA MANAGEMENT RESPONSIBILITIES

The responsibilities designated to each member of the research team are documented on the Delegation of Authority Form. The study coordinator and research assistants will be responsible for the informed consent

process, review for eligibility, questionnaire administration, data entry, device administration, and CRF entries. The study coordinator will be responsible for AE/SAE documentation and reporting, while the PI will be responsible for the AE assessment, review of the AE documentation forms, and overview of the research staff.

## 12.5 DATA CAPTURE METHODS

Data will be entered directly from the source documents and stored on a password-protected computer in the Frohlich Lab by a researcher. All stored data will be de-identified and will not contain any reference to participant name or other personal information, such as email or phone number.

## 12.6 PROTOCOL DEVIATIONS

All deviations from the protocol will be addressed in study participant source documents. The researcher will complete a Protocol Deviation Log using the participant code as the identifier. This form will collect information such as the date the deviation occurred, details of what the deviation consisted of, any corrective and preventative actions that were taken as a result of the deviation, and the date that the PI and IRB were notified. The PI will review the information and initial once approved. A completed copy of the Protocol Deviation Form will be maintained in the regulatory file, as well as in the participant's source document. Protocol deviations will be sent to the IRB per their guidelines. The site PI/study staff will be responsible for knowing and adhering to their IRB requirements.

## 12.7 RECORD RETENTION

According to the University of North Carolina at Chapel Hill's Archives and Record Management Services schedule for General Records Retention and Disposition Schedule 6.10, records will be kept for 5 years after the completion of the study or grant end date, whichever is later.

# 13 ETHICAL CONSIDERATIONS

## 13.1 ETHICAL STANDARD

The investigator will ensure that this study is conducted in full conformity with the principles set forth in the Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research, as drafted by the US National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR Part 46 and/or the ICH E6; 62 Federal Regulations 25691 (1997).

## 13.2 INSTITUTIONAL REVIEW BOARD (IRB)

The Office of Human Research Ethics is responsible for ethical and regulatory oversight of research at UNC-Chapel Hill that involves human participants. The OHRE administers, supports, and guides the work of the Institutional Review Boards and all related activities. Any research involving human participants proposed by faculty, staff, or students must be reviewed and approved by an IRB before research may begin, and before related grants may be funded. OHRE and the IRBs are critical components of the coordinated Human Research Protection Program, which serves to protect the rights and welfare of human participants. All components of this program must work together to ensure institutional compliance with ethical principles and regulatory requirements. The following is a mission statement for the coordinated Human Research Protection Program:

The University of North Carolina at Chapel Hill is committed to expanding and disseminating knowledge for the benefit of the people of North Carolina and the world. An important part of that commitment to knowledge is research of the highest quality on all aspects of the health and behavior of people, and such research is only possible through the participation of humans as research participants. Human participants are partners in research and a precious resource to the university. At UNC-Chapel Hill, human participant research is a privilege, but not a right. Consistent with that philosophy, it is the mission of the UNC-Chapel Hill Human Research Protection Program to ensure that:

a. The rights and welfare of human participants are paramount in the research process;
b. The highest standards of ethical conduct are employed in all research involving human participants;
c. Research investigators are properly trained in the ethical and regulatory aspects of research with human participants;
d. Research investigators deal honestly and fairly with human participants, informing them fully of procedures to be followed, and the risks and benefits of participating in research; and
e. Research using human participants at UNC-CH conforms to applicable local, state, and federal laws and regulations and the policies of the university.

## 13.3 INFORMED CONSENT PROCESS

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Extensive discussion of risks and possible benefits of tACS will be provided to the participants. Consent forms describing, in detail, the study intervention, device, procedures, and risks are given to the participant and written documentation of informed consent is required prior to the administration of any treatment or assessments used in this study. All consent forms will be IRB-approved and updated with any new information as modifications are made throughout the study.

The researcher and potential participants will review the clinical trial in its entirety by reviewing the consent form together in a private location. If the participant is unclear on any part of the consent form, the research will return

to the section and explain further. Participants must sign the informed consent document prior to any procedures taking place. Participants may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the participants for their records.

## 13.4 EXCLUSION OF WOMEN, MINORITIES, AND CHILDREN (SPECIAL POPULATIONS)

Non-English speaking individuals are excluded because the ability to accurately and complete communicate study information, answer questions about the study, and obtain consent is necessary. Female participants will be asked if there is any reason to believe they might be pregnant. Pregnant participants will be excluded despite the fact that theoretical risk to mother or fetus is exceedingly small, since no safety data for pregnancy is known to exist for transcranial current stimulation studies. All women of child-bearing potential will be asked to take a pregnancy test during the initial session in order to determine eligibility for the study.

## 13.5 PARTICIPANT CONFIDENTIALITY

Participant confidentiality is strictly held in trust by the participating investigators, their staff, and the research team. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants.

All data will only be referenced by dummy identifier code. Data will be stored on a password protected computer. A key connecting names and code numbers will be kept in a locked cabinet, accessible only by research personnel. All data will be stored and analyzed on password protected computers, also only accessible by research personnel. Participants will not be identified in any report or publication about this study. See *10 Data Handling and Record Keeping* for more information on source documentation storage and security.

## 13.6 STUDY DISCONTINUATION

In the event that the study is discontinued, participants who have completed or who are still enrolled in the study will be notified. Any new information gained during the course of the study that might affect participant's willingness to continue will be communicated within 2 days of the PI learning this information.

# 14 PUBLICATION POLICY

This study will be registered on clinicaltrials.gov once IRB approved. There are no restrictions on publications since this is an investigator-initiated study funded by a grant agency (NARSAD) that has no influence on the publications resulting from this study. The aim is to publish the results of this study in a peer-reviewed, highly-ranked psychiatry journal.

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## APPENDIX A: SCHEDULE OF EVENTS

| **Procedures** | Phone Screening | Day 1 of Stimulation | Day 2 of Stimulation |
|---|---|---|---|
| Provide Verbal Consent | x | | |
| Signed Consent Form | | x | |
| Assessment of Eligibility Criteria | x | x | |
| Urine Pregnancy Test | | x | |
| Urine Drug Test | | x | |
| Baseline Assessments (PSS, CD-RISQ, PSQI) | | x | |
| Saliva Sample | | x | x |
| Stimulation | | x | x |
| Stimulation Questionnaire | | x | x |
| Saliva Sample | | x | x |
| Final Questionnaires (PANAS, STAI) | | x | x |

## APPENDIX B: AE REPORT FORM

**Adverse Effects Report:**

*Reasons for Report (adverse event, time, date and place of occurrence if available)*:

1. What do we already know about the therapy?
    a.
2. What is the temporal relationship of the AE to the study therapy?
    a.
3. Does the AE improve or disappear when the therapy is stopped?
    a.
4. Is the AE a worsening of baseline symptom(s)?
    a.
5. Is the AE a result of an underlying concurrent medical condition(s) or concurrent medication(s)?
    a.
6. Additional Information provided by research team
    a.

Research team member signature _____

Date_____

Co-Investigator:

_____
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Steps to be taken (if applicable)

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Co-I signature

_____Date_____

PI Comments:

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Steps to be taken (if applicable)

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PI signature

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