Mechanism of Action of Transcranial Alternating Current Stimulation for the Treatment of Major Depressive Disorder

NCT number NCT03994081
Document Date 05/02/2022

Mechanism of Action of Transcranial Alternating Current Stimulation for the Treatment of Major Depressive Disorder

Protocol Number: 20-1822

Principal Investigator: David Rubinow, MD

Co-Investigator: Flavio Frohlich, PhD

Co-Investigator: Crystal Schiller, PhD

Funded by: Foundation of Hope

Version Number: 2

02 May 2022

Summary of Changes from Previous Version:

Affected Section(s)	Summary of Revisions Made	Rationale
1.2	Updating study schema	We deleted unnecessary administration of questionnaire to increase feasibility and reduce burden for the participant.
1.3	Updating of Schedule of Activities (SoA)	Now, the SoA provides accurate and clear information over all study procedures.
8.2	No YMRS ratings during the stimulation week	Signs of Mania are assessed during Screening, at Day 1, Day 5 and follow-up to ensure safety. The assessment during D2-D4 prolongs the experimental session without a significant gain in safety.
1.1, 4.1	We deleted the MRI study visit	With regards to feasibility, we opted against the use of MRI and deleted it from the Master Protocol. The MRI was initially not included for safety purposes but as potential correlative research outcome.
5.5	Use of non-encrypted emails	Potential participants often did not respond to encrypted email. We hope to foster recruitment by using standard emails. No medical data is inquired in any outgoing email and all medical data is collected within a HIPAA conform Zoom call or via RedCap.
5.5	Recruitment procedure, RedCap Screening, Pre-Screening (remotely)	We slightly restructured out recruitment process to facilitate recruitment and simply the process for our participants.
6.3, 9.4.6	Updated Randomization Personnel	Addressing personnel changes in our lab.
1.1, 4.1	Adjusted total hours for study	The Master Protocol is now matching the actual study duration.
1.1	Electronic informed consent / HIPAA consent	We like to cover all parts of our study optimally by informed consent. Since the first two sessions are held remotely via phone/Zoom, we opt for an electronic consent obtained during the first session (pre-screening). A paper copy of the consent is handed to the participant within the first stimulation session.
9.4.6	We do not plan an interim analysis	Results would be flawed by low number of participants
5.2	We reduced the necessary duration of stable antidepressant medication from 6 to 4 weeks.	A clinical benefit after initiating an antidepressant treatment is expected after 2-4 weeks. We therefore demand a stable (or none) antidepressant medication for at least 4 weeks. Note that this does not demand the patient to initiate a new pharmacological treatment but requires a stable dose on his current medication.
5.2	We specified the exclusion criteria related to alcohol and substance use. Now, moderate and severe alcohol/substance use disorder are defined as exclusion criteria.	We specified the diagnosis auf AUD and substance use disorder based on DSM-V and present now a clear cut off to exclude participants.
10.1.6, 8.3.5	Since the inclusion of the DSMB is not necessary for this study, we deleted the phrases related to that institution.	Adapting the Master Protocol to reflect the actual experiment.
7.1.	We specified what happens if a subject withdraws from the study during the stimulation period.	We will try to schedule the 2-week follow-up to administer the respective questionnaires. This is assessment is related to research purpose only.
5.1.	Inclusion age changed to 18-70 years	Represent a broader spectrum of society.

Table of Contents

STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all subject materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any subject is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from subjects who provided consent, using a previously approved consent form.

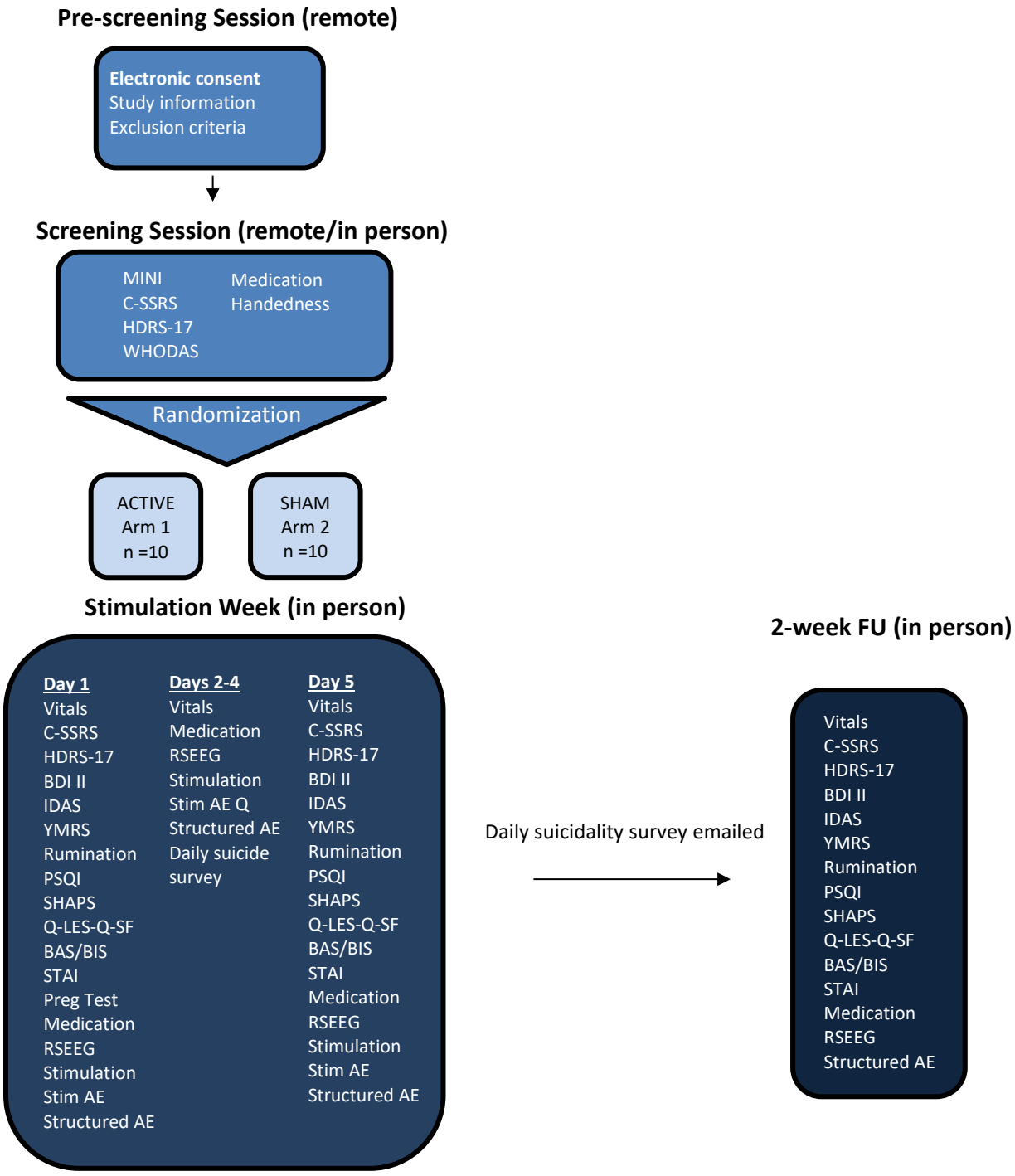
1. PROTOCOL SUMMARY

1.1 SYNOPSIS

Title:	Mechanism of Action of Transcranial Alternating Current Stimulation for the Treatment of Major Depressive Disorder
Study Description:	The purpose of this clinical trial is to investigate the mechanism of action of non-invasive transcranial alternating current stimulation (tACS) in subjects with major depressive disorder (MDD). We will recruit 20 males and females with unipolar, non-psychotic MDD. Eligible subjects will have 5 consecutive 40-minute stimulation sessions. Subjects will be randomly assigned to one of two groups: sham stimulation or 10Hz (alpha) tACS. Participation will include 8 visits, two of them remotely. Participants will give electronic informed consent during the first remote screening sessions. At the initial stimulation session (D1), the electronic consent is printed out and handed to the participant. Eligible subjects will undergo 5 consecutive stimulation sessions with ongoing assessment of suicidality and manic symptoms. Subjects will fill in a questionnaire about stimulation side effects. Neurophysiological measures with high-density EEG will be taken before and after every stimulation session, as well as at the 2-week follow-up visit. Clinical assessments will be performed at baseline (D1), Day 5 of stimulation, and at the follow-up visit using the Hamilton Depression Rating Scale (HDRS-17). Additional assessments of symptomology, quality of life, sleep, and arousal will be completed per the research schedule and are mostly implemented as electronic surveys.
Objectives:	Primary Objective: To investigate the physiological changes in subjects with MDD over the course of a 5-day, 40-minute stimulation protocol, specifically changes in alpha oscillation power from resting state EEG recordings over the course of the intervention (D1 to D5 of stimulation, to the follow-up visit). Secondary Objectives: To elucidate the relationship between changes in EEG and changes in depressive symptoms, by comparing the changes in clinical assessments (e.g., HDRS) and the change in alpha oscillation power over the course of the intervention (baseline to day 5 of stimulation, to the follow-up visit).
Endpoints:	Primary Endpoint: Immediate effects on resting state EEG recordings on each day of stimulation as well as long term effects 2-weeks after stimulation. Secondary Endpoints: Relationship between symptoms of depression per self-report and clinician rated and physiological changes.
Study Population:	We will recruit 20 males and non-pregnant females (age 18-70) with a diagnosis of unipolar, non-psychotic MDD, free of benzodiazepines and anticonvulsant medications. Eligible subjects will have a Hamilton Depression Rating Scale (HDRS-17) > 8 and low suicide risk (determined by scoring less than 3 on the Suicide Item on the HDRS as well as having no active intent as determined by the Columbia Suicide Severity Rating Scale

	(C-SSRS). Subjects will be recruited from the Chapel Hill, Durham and Raleigh areas.
Phase:	N/A
Description of Sites/Facilities Enrolling Participants:	University of North Carolina at Chapel Hill.
Description of Study Intervention:	We will the XCSITE100 stimulator designed by PulvinarNeuro for investigational purposes to deliver either active sham or 10Hz transcranial alternating current stimulation. Active sham treatment will include 20 seconds of ramp in to 40 seconds of 10Hz tACS with a ramp out of 20 seconds for a total of 80 seconds of stimulation. The choice of an active sham is motivated to enhance success of subject blinding by mimicking skin sensations associated with tACS. 10Hz tACS will also have a 20 second ramp in and ramp out with 40-minutes of stimulation for a total of 2440 seconds. Stimulation waveforms are sine-waves with a zero-to-peak amplitude of 1mA. Subjects will stay in a relaxed yet experimentally controlled state by watching a nature movie such as “Reefscape” during stimulation.
Study Duration:	2 years
Participant Duration:	Participation for each subject will last no longer than 2-3 weeks total. Completion will involve a maximum number of 8 appointments. The initial session is a remote (via Zoom) pre-screening. Participants are asked general exclusion criteria regarding brain stimulation studies and describe their mental health history. The meeting takes approx. 30 minutes. If no exclusion criteria are present, an appointment for the screening session is scheduled. This session – in person or remotely – involves the clinical assessment of depression severity (HDRS), psychiatric diagnose (M.I.N.I.) and suicidality (C-SSRS). After consultation with the study PI Dr. Rubinow, treatment resistance is determined (Maudsley Staging Method) and participants are randomized and start the five-day stimulation procedure. The initial session (D1) and D5 will take approximately 4 hours. D2-D4 will take 90 minutes. The 2-week follow-up will take approximately 3 hours. We estimate total participation time to be approximately 17 hours.

1.2 SCHEMA


Pre-screening Session (remote)
Electronic consent
Study information
Exclusion criteria
Screening Session (remote/in person)
MINI
C-SSRS
HDRS-17
WHODAS
Medication
Handedness
Randomization
ACTIVE
Arm 1
n =10
SHAM
Arm 2
n =10
Stimulation Week (in person)
Day 1
Vitals
C-SSRS
HDRS-17
BDI II
IDAS
YMRS
Rumination
PSQI
SHAPS
Q-LES-Q-SF
BAS/BIS
STAI
Preg Test
Medication
RSEEG
Stimulation
Stim AE
Structured AE
Days 2-4
Vitals
Medication
RSEEG
Stimulation
Stim AE Q
Structured AE
Daily suicide survey
Day 5
Vitals
C-SSRS
HDRS-17
BDI II
IDAS
YMRS
Rumination
PSQI
SHAPS
Q-LES-Q-SF
BAS/BIS
STAI
Medication
RSEEG
Stimulation
Stim AE
Structured AE
Daily suicidality survey emailed
2-week FU (in person)
Vitals
C-SSRS
HDRS-17
BDI II
IDAS
YMRS
Rumination
PSQI
SHAPS
Q-LES-Q-SF
BAS/BIS
STAI
Medication
RSEEG
Structured AE

1.3 SCHEDULE OF ACTIVITIES (SOA)

Procedures	Pre-Screening (remote)	Screening (in person / remote)	Day 1 of Stimulation (D1)	Day 2 of Stimulation (D2)	Day 3 of Stimulation (D3)	Day 4 of Stimulation (D4)	Day 5 of Stimulation (D5)	Interim Day 6-Day 21 +/-3 days	2-week Follow up (FU) Day 22 +/-3 day s
Electronic Informed consent (S)	X								
HIPPA authorization			X						
Medication & Psychotherapy	X	X	X	X	X	X	X		X
Determine Eligibility	X	X							
Handedness (S)		X							
Demographics + Medical Hx (S)		X							
M.I.N.I.		X							
C-SSRS		X	X				X		X
HDRS-17		X	X				X		X
WHODAS 2.0 (S)		X							
Maudsley Staging Method		X							
Randomization		X							
Urine Pregnancy Test			X						
Beliefs about Treatment (S)			X						
IDAS (S)			X				X		X
Rumination (S)			X				X		X
SHAPS (S)			X				X		X
PSQI (S)			X				X		X
BDI II (S)			X				X		X
Q-LES-Q-SF (S)			X				X		X
BAS/BIS (S)			X				X		X
STAI Y1 / Y2 (S)			X				X		X
YMRS			X				X		X
Vitals			X	X	X	X	X		X
EEG			X	X	X	X	X		X
tACS Stimulation (alpha/sham)			X	X	X	X	X		
Daily Stimulation Q (S)			X	X	X	X	X		
AE Structured Interview + Review			X	X	X	X	X		X
Blinding Q (subject) (S)							X		
Blinding Q (operator) (S)							X		
Symptom Improvement Q. (S)							X		
Daily Suicide Survey (S)			X----------				----------X	X----------	X

(S): implemented as survey in RedCap, Q is used as abbreviation for questionnaire.
Daily Suicide Surveys will be conducted in RedCap throughout the study

2. INTRODUCTION

2.1 STUDY RATIONALE

Major depressive disorder (MDD) is a common, severe psychiatric illness that affects 6.7% of adults every year in the United States [1]. MDD is characterized by depressed mood, loss of interest or pleasure in activities that used to be enjoyable (anhedonia), sleep disturbances, cognitive impairment, and other symptoms that can severely affect quality of life. Current recommended pharmacological treatments are non-targeted, resulting in undesirable side effects. Furthermore, the STAR*D trial demonstrated that a large percentage of patients are treatment-resistant, and that remission is less likely following multiple drug trials[2]. More effective and safer therapies for the treatment of MDD are desperately needed.

2.2 BACKGROUND

MDD has been associated with hypoactivity in the left dorsolateral prefrontal cortex [3], characterized by elevated alpha oscillations [4]. Therefore, targeting and reducing alpha oscillations in the left dorsolateral prefrontal cortex could prove beneficial to patients with MDD. Targeted stimulation modalities, such as transcranial alternating current stimulation (tACS), can directly engage and modulate oscillatory activity. TACS utilizes sine-wave stimulation waveforms that can enhance specific oscillatory activity [5]. The proposed study is a follow-up to a recently concluded trial run by the Carolina Center for Neurostimulation under the direction of Drs. Frohlich and Rubinow [6].

This recently published trial compared the efficacy of 10Hz-tACS (therapeutic frequency, n = 10), 40Hz-tACS (control frequency, n = 10), and sham/placebo stimulation (n = 10). The intervention consisted of 5 consecutive days of 40-minutes of stimulation. Results demonstrated that 2-weeks after completion of the intervention, the 10Hz-tACS group had more subjects responding to treatment (i.e., at least a 50% decrease in depressive symptoms) as measured by the clinician-administered assessments (Montgomery-Asberg Depression Rating Scale, $p = 0.026$, and Hamilton Depression Rating Scale, $p = 0.026$). Concurrently, we found that 10Hz-tACS successfully reduced alpha oscillations in the left frontal regions after completion of the stimulation protocol ($p < 0.05$). While these data demonstrate preliminary efficacy, it is unclear how tACS caused this change, indicating a need to elucidate neurophysiological mechanism of action. Previous work from other groups demonstrate an increase in alpha oscillations following tACS (e.g., [[7]–[9]]), whereas our stimulation paradigm resulted in a decrease in alpha oscillations. Importantly, what is missing is the temporal course of these physiological changes and how they correspond to clinical outcomes. By including high-definition EEG before and after each stimulation, we will be able to delineate the neurophysiological reorganization of alpha oscillations in response to repeated application of tACS.

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 KNOWN POTENTIAL RISKS

Risk of Confidentiality Breach: In the unlikely event of a breach of confidentiality, people might discover that an individual was involved in this research study. This is especially sensitive because the clinical population recruited for this study may be subjected to negative consequences caused by the stigma of mental disorders. Furthermore, some might not agree with the principle of participating in research or of

changing natural brain activity. To avoid breaches in confidentiality, study documents that contain personal information, including the informed consent document, and the document that links study ID numbers to personal identifying information are kept in locked filing cabinets in locked rooms, separate from any source documents containing subject dummy identifiers. All data is stored in locked cabinets inside locked offices; electronic data will be stored only on password-protected computers, and data encryption methods will be used during communication between investigators. Interviews will be conducted over the HIPAA approved Zoom account. Only study personnel will have access to the data. All study staff participate in annual human subject training that includes education about responsibilities to the minimize risk of confidentiality breach.

Risk of Embarrassment: Self-report assessments contain questions regarding sensitive personal information. This risk is necessary in order to assess mood symptoms and associated psychopathology. Subjects will be assured upon intake that only study personnel will see any clinical ratings and that self-identifying information will not be collected alongside HIPAA protected information.

Risk of Injury and Discomfort: The side effects of tACS are mild and transient; in fact, low intensity transcranial current stimulation, such as tACS, has been used for several years without any report of serious side effects [10]. Furthermore, this stimulation mode 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 [11]. However, tACS does have some mild side effects, such as transient mild tingling, burning, or itching under the electrode sites. In our previous trial, subjects from all three groups of stimulation reported either absent or mild side effects, and there was no difference between the groups with the exception of “flickering lights” (or phosphenes, $p = 0.014$)[6]. To monitor these mild side effects, we will be administering a stimulation questionnaire after each stimulation session to determine whether these effects were experienced and at what intensity. Research personnel is present during the full experimental. If subject is experiencing severe discomfort (as determined by the questionnaire or by self-report), the stimulation will be stopped immediately.

Patients with MDD have an about 20 times higher rate of suicide than average. We have no evidence that our treatment paradigms will in any way increase this likelihood. In the previous iteration of this trial, 4 subjects in the sham/placebo stimulation group experienced an increase in suicidal thoughts and only one of those 4 subjects reported suicidal intent [6]. No subjects who received tACS reported an increase in suicidal ideation from baseline. Regardless, subjects with high suicide risk will not be included in this study. If an enrolled subject shows signs of suicide risks that were not apparent during enrollment, a referral to UNC Psychiatry will be made. Dr. Schiller, Co-I, will facilitate this process.

We will be using the Suicide Item included in the HDRS-17 [12] to assess suicide risk, as well as the Columbia Suicide Severity Rating Scale (C-SSRS) [13] to determine intent for suicide during the screening interview. Inclusion criteria state that the subject must be low suicide risk and that potential subjects with an above “low risk” designation will not be eligible for the study. After the subject enrolls in the study, we will evaluate suicidal thoughts daily through an online questionnaire administered through REDCap. Additionally, the C-SSRS will be administered at D1, D5, and at the 2-week follow-up visit. In the event that suicide risk increases during participation in the study, the subject will be asked to stop the study. The subject will be provided with a referral to UNC Department of Psychiatry, and their mental health care or family medical doctor will be contacted.

2.3.2 KNOWN POTENTIAL BENEFITS

This study has not been designed to benefit the individual subjects. However, subjects in this study may experience some degree of relief from mood symptoms because of the tACS treatment. Our last trial revealed preliminary efficacy, as participants who received 10 Hz tACS stimulation showed a 50% decrease in symptoms of depression two weeks later [6].

2.3.3 ASSESSMENT OF POTENTIAL RISKS AND BENEFITS

The risks and benefits presented above are no more serious than for other clinical trials in this population. Based on the need for complementary and alternative treatments for MDD, the potential risks are worth the potential benefits. Justification as to why the risks of participation in the study outweigh the value of the information to be gained.

3. OBJECTIVE AND OUTCOME MEASURES

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
To determine the effect of 10 Hz tACS on brain physiology as measured with electroencephalogram (EEG).	Resting state EEG recordings taken before/after every stimulation session, as well as at a 2-week follow up visit.	The focus is to determine the mechanism of action for five consecutive days of forty-minute tACS stimulation sessions. These endpoints will allow the examination of both immediate and long-term effects of 10 Hz tACS on physiology.
Secondary		
To elucidate the relationship between change in physiology and change in clinical symptoms	Correlations between Hamilton Depression Rating Scale (HDRS), Beck Depression Inventory (BDI) and alpha oscillation power (as measured by resting state EEG recordings).	Previous work indicates that 10 Hz tACS modulates mood and physiology, but there is no clear indication that these changes are related.

4. STUDY DESIGN

4.1 OVERALL DESIGN

The design for this study is a pilot, randomized, double-blind, sham-controlled, clinical trial which will be used to investigate the mechanism of action of 10 Hz tACS in subjects with MDD, as well as determine the relationship between physiological changes and clinical changes. We are recruiting from a clinical population. Participants will be 20 males and non-pregnant females ages 18-70 with unipolar, non-

psychotic MDD, free of benzodiazepine and anticonvulsant medication, who have a HDRS-17 > 8 and are at a low risk for suicide according to the Hamilton Depression Rating Scale as well as the Columbia Suicide Severity Rating Scale (C-SSRS). All women of child-bearing potential will be asked to take a pregnancy test during the initial session to determine eligibility for the study; nursing or pregnant subjects will be excluded from participation, and all women of child-bearing potential will be required to use an appropriate form of birth control throughout their participation. These individuals will be outpatients and may or may not seek mental health care from a family practitioner, therapist, or psychiatrist.

This is a single-site, pilot clinical trial with 2 arms. Due to the Covid pandemic recruitment was slower than expected. We anticipate to complete study enrollment until 12/2022. Subjects will be randomly assigned to one of two arms: active sham stimulation or 10 Hz (alpha) tACS. We will use the XCSITE100 stimulator designed by PulvinarNeuro for investigational purposes to deliver stimulation over five consecutive days. Subjects will stay in a relaxed yet experimentally controlled state by watching a nature movie such as "Reefscape" during stimulation. The initial session (D1) and D5 will take approximately 4 hours. D2-D4 will take 90 minutes. The 2-week follow-up will take approximately 3 hours. We estimate that total participation to be approximately 17 hours.

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

This study is a double-blind, randomized, sham-controlled interventional study. The choice of double blind and randomization is important for the integrity of our data, especially for the clinical assessments. All individuals involved with data collection (as well as all randomized subjects) will be blind to the subject's arm assignment until all data has been collected. This will reduce implicit and explicit bias in the data collection process. In this study, subjects will be randomized in to one of two arms: active sham (i.e., placebo) stimulation or 10 Hz tACS. This is a follow-up to a previous study that compared 40 Hz tACS (control frequency), 10 Hz tACS and sham/placebo stimulation. The greatest effect was found when comparing 10 Hz tACS and sham/placebo stimulation; no effect was found with 40 Hz tACS. Furthermore, for 40 Hz tACS the study blind was compromised by stimulation-induced phosphenes, with 80% of subjects correctly guessing that they had received stimulation. As a result, we choose to further explore 10 Hz tACS in comparison to sham/placebo stimulation, omitting 40 Hz tACS as our control frequency. While having only a single control (placebo/sham) may be problematic, we have determined that our smaller, second part of this study does not require the same 3 arms as previously used, as the primary outcome is target engagement measured with the EEG and to further understand the mechanism of action of tACS in a multi-day study. In this design, our sham/placebo stimulation is active, including 80 seconds of stimulation (20 seconds ramp-in to 40 seconds of 10 Hz tACS at 1 mA with 20 seconds of ramp-out). This choice of active sham/placebo is important to mimic the skin sensations and other transient side effects associated with stimulation. Typically, stimulation side effects only last approximately a minute into stimulation; indicating that theoretically sham/placebo stimulation should be difficult to differentiate from 10 Hz tACS.

4.3 JUSTIFICATION FOR DOSE

As a continuation study, we are utilizing the exact same dosage as given before: 5 consecutive days of 40-minutes of stimulation [6]. This dosage was chosen based on a previous study, which examined transcranial direct current stimulation (tDCS) for the treatment of MDD [14].

4.4 END OF STUDY DEFINITION

The end of this study is defined as when the last subject completes the final study visit (i.e., the 20th subject completes their 2-week follow-up visit).

5. STUDY POPULATION

5.1 INCLUSION CRITERIA

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

- Ages 18-70 years
- DSM-IV diagnosis of MDD; unipolar, non-psychotic
- Hamilton Rating Depression Rating Scale (HRDS-17) score >8
- Low suicide risk as determined by a score of <3 on the Suicide Item on the HDRS-17 and based on additional information from the C-SSRS (no intent)
- Capacity to understand all relevant risks and potential benefits of the study (informed consent)

5.2 EXCLUSION CRITERIA

An individual who meets any of the following criteria will be excluded from participation in this study:

- DSM-V diagnosis of moderate or severe alcohol use disorder (AUD) within the last 12 months.
- DSM-V diagnosis of moderate to severe substance use disorder (excluding tobacco) within the last 12 months.
- Current axis I mood, or psychotic disorder other than major depressive disorder
- Lifetime comorbid psychiatric bipolar or psychotic disorder
- Eating disorder (current or within the past 6 months)
- Obsessive-compulsive disorder (lifetime)
- Post-traumatic stress disorder (PTSD, current or within the last 6 months)
- Attention deficit hyperactivity disorder (ADHD, currently under treatment)
- Current use of benzodiazepines or anti-epileptic drugs
- Antidepressant drugs taken for less than 4 weeks (i.e., recently initiated)
- Neurological disorders, including but not limited to history of seizures (except childhood febrile seizures and ECT-induced seizures), dementia, history of stroke, Parkinson's disease, multiple sclerosis, cerebral aneurysm.
- Medical or neurological illness (unstable cardiac disease, AIDS, malignancy, liver or renal impairment) or treatment for a medical disorder that could interfere with study participation; comorbid neurological condition (i.e. seizure disorder, brain tumor)
- History of traumatic brain injury that required subsequent cognitive rehabilitation, or cause cognitive sequelae.
- Prior brain surgery and/or any brain devices/implants, including cochlear implants and aneurysm clips
- Current pregnancy or lactation. If the ability to become pregnant exists, unwillingness to use appropriate birth control measures during study participation

- 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
- Non-English speakers

5.3 LIFESTYLE CONSIDERATIONS

Benzodiazepine use will be prohibited during this study, unless used as needed. If subjects are prescribed benzodiazepines as needed (PRN), they will be requested not to use benzodiazepines within 48 hours of any study session. Current medication intake is queried every day during the stimulation period.

5.4 SCREEN FAILURES

In the design of this study, initial phone or zoom screening procedures should identify the majority of subjects who could potentially become screen drop outs in the clinical trial. However, the phone/zoom screening process does not necessarily account for all exclusion criteria.
In the case that a subject enrolls to participate in the trial and the first interview reveals that they do not meet study criteria, the study personnel completing the interviewing process will clearly explain why the subject does not meet criteria. However, in the case that a subject does not qualify based on suicide risk, procedures will be followed to ensure subject safety.

5.5 STRATEGIES FOR RECRUITMENT AND RETENTION

This clinical trial will utilize multiple recruitment strategies to communicate this opportunity to as many potential participants as possible. Our first means is through a referral process. Participants can be referred to the study through their primary mental health care provider or family doctor during routine visits. This type of recruitment will take place in doctors' offices throughout Chapel Hill, Carrboro, Durham and Raleigh areas. We estimate that approximately 15 participants will be enrolled from the Chapel Hill/Carrboro area and 5 between Durham and Raleigh. Clinicians will be informed of inclusion criteria through email and listserv announcements and be asked to mention this clinical trial to appropriate patients and offer them a flyer/ brochure with contact information. We will also be using the UNC i2b2 to send request forms to the Carolina Data Warehouse to recruit participants who have been seen at UNC Hospitals that meet the inclusion criteria. [1]

In addition to referrals through primary care providers, we will advertise the study directly to the public on websites such as ClinicalTrials.gov, studypages.com, Research For Me, frohlichlab.org and Carolinaneurostimulation.org. We will have contact information and a summary of the clinical trial posted on the Frohlich Lab Facebook and Twitter pages. Will we also be launching a Facebook or Instagram advertisement to identify potential patients. This advertisement will include a link to a brief screening survey on RedCap to help identify participants. We will also be using the UNC Mass email and department listserv to send out an email that has the link to the survey. All patient identifiers will be stored in REDCap

until recruitment is over. When recruitment is over, all patients who do not consent or are not eligible for participation in the study will have their responses permanently deleted in REDCap.

We will send unencrypted emails to facilitate the initial contact to potential participants. Medical information is never requested per email. All medical information is recorded through HIPAA conform Zoom meetings and RedCap surveys.

Our retention strategy includes a payment schedule of three times per subject. The subject will receive payment in form of a Visa Card at D1 ($75). This card will be charged again on D5 ($140), and the final follow-up session ($40). Thus, completion of the study will result in a financial compensation of $255.

Research personnel will be easily available for the subjects to contact via email or phone. The inclusion criteria state that each subject must be able to understand all risks and benefits associated with this study. We will be asking each subject to answer questions about the consent form to determine that the study process and the duration of participation are completely understood by all subjects. We will aim to have a specific research team member assigned to complete all sessions with the same subject. However, we will not require the same researcher to be present during stimulation sessions 2 through 4. The study team will work hard at forming rapport with the subject so they feel comfortable and willing to discuss what may be sensitive information. Retention will be quantified by the fraction of subjects coming to each scheduled session (the data from each session will be scored and documented the day of the session).

As this study requires several days of participation in a row, we will allow subjects to miss one stimulation session, if it is made up at the end of the stimulation week. If two consecutive stimulation sessions are missed, the subject will be withdrawn from participation. This schedule would permit the subject to still receive the full intervention (5 days of stimulation) without being withdrawn from the study due to unforeseen circumstances (e.g., inclement weather, car trouble). In addition, the 2-week follow-up session will be scheduled on the first day of enrollment to allow for planning but within ± 3 days of the final day of stimulation to account for possible scheduling problems.

6. STUDY INTERVENTION

6.1 STUDY INTERVENTION(S) ADMINISTRATION

6.1.1 STUDY INTERVENTION DESCRIPTION

We will be using the XCSITE100 stimulator designed by PulvinarNeuro in the Frohlich Lab for investigational purposes. The device is not implanted and has not been designed for or 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 subject. 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 the XCSITE 100 and comparable devices (i.e., the commercial, CE-certified Neuroconn Plus stimulator) have been conducted under an IDE and 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. While the Neuroconn device is commercially

available, it cannot be preprogrammed for 10Hz-tACS, and is not designed for tACS clinical trials. This makes the use of the Neuroconn device not appropriate for this study.

The XSCITE100 stimulator may apply 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. tACS may be applied for currents between 100 µA and 2 mA (peak-to-peak for tACS). For the purposes of this study, this device will be set to deliver either sham stimulation or 10Hz-tACS. The XCSITE100 device runs on a single 9V rechargeable battery. The device itself is run through a device on a tablet. For more instructions, please see the submitted instruction manual.

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

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 $\pm$3 mA peak. If the current exceeds these limits, 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 $\pm$ 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
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 blow, and the stimulator will no longer be electrically connected to the device.
4. *Power supply fuse.* Finally, if for any 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.

6.1.2 DOSING AND ADMINISTRATION

The research team will first measure each subject's head using the 10-20 system to determine the electrode locations. Subjects will then be fitted with the 3 electrodes for stimulation: two 5x5cm electrodes placed over F3 and F4, and one 5x7cm electrode placed over Cz. Electrodes will be carbon rubber, with Ten20 conductive paste applied. During stimulation, the subject will be in a relaxed and experimentally controlled state by watching a nature movie (such as Reefscapes). One session of stimulation will be performed per day, for 40-minutes. Before and after each stimulation session, an eyes open and eyes closed resting state EEG will be performed.

The stimulation waveforms used in this study are sham/placebo stimulation and 10 Hz tACS at 1 mA zero-to-peak amplitude. Sham/placebo stimulation includes 20 seconds of ramp-in to 40 seconds of 10 Hz tACS at 1 mA to 20 seconds of ramp-out, for a total of 80 seconds of stimulation. This is intended to mimic the skin sensations (e.g., itching, burning, tingling) that are experienced at the onset of stimulation, assisting with blinding the subject's assignment. 10 Hz tACS also has a ramp-in and ramp-out of 20 seconds, with 40-minutes of stimulation at an amplitude of 1 mA, for a total of 2440 seconds of stimulation.

Stimulation devices will be pre-programmed, and codes will be randomized to one of the two experimental arms. Researchers will enter the subject-specific code into the tablet that controls the XCSITE100 device and will monitor subjects during the 40-minutes of the stimulation. Research personnel will be thoroughly trained and have trainings documented on the transcranial stimulation device and will be present during all stimulation sessions. To monitor side effects of stimulation a stimulation effects questionnaire will be administered after each stimulation session.

6.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

Randomization
Dr. Mengsen Zhang, a Frohlich Lab member, will randomize 20 6-digit codes, which will be used by the study coordinator and research assistants. These codes are directly linked to which treatment subjects receive (sham/placebo or 10 Hz tACS at 1 mA) and will be entered into the XCSITE tablet. The assignment of each subject cannot be determined by looking at the codes (e.g., codes are not sequential, code assignment is not based on “odd” or “even” numbers). Dr. Mengsen Zhang has no other responsibility in the study other than providing these randomized codes. If Dr. Mengsen Zhang leaves the Frohlich Lab, another equivalent researcher who does not work with human subjects will perform this task.

Blinding
The study is double-blind. This means that the subject and the researchers are unaware of each subject's assignment until the completion of all data collection. This is accomplished using the randomization codes described above. Furthermore, this study utilizes an active sham stimulation. This means that the sham/placebo condition includes some stimulation, mimicking the skin sensations associated with tACS. Blinding success is tested on D5 with two separate questionnaires for operator and participant. In our

previously concluded trial, subjects in the sham and 10 Hz tACS groups responded similarly to the blinding questionnaire, indicating that our active sham stimulation successfully blinded the subjects.

6.4 STUDY INTERVENTION COMPLIANCE

Full compliance with the intervention is defined as completing all 5 daily stimulation sessions for 40 minutes each day. As the intervention is applied and monitored by research personnel, compliance can be directly observed.

6.5 CONCOMITANT THERAPY

Eligible subjects will be permitted to be receiving concomitant therapy, such as psychotherapy, antidepressants, or other medications. The only medications not permitted during this trial are anticonvulsants and recent benzodiazepines. The use of antidepressants or other therapies may affect our outcome, as subjects may experience relief of their depressive symptoms from these treatments. However, eligibility for this trial includes stable medication for the past 4 weeks. An adequate dose of antidepressants includes at least 4 weeks of use, so we anticipate that this requirement will reduce the potential bias for permitting the use of antidepressants and other medications. We also aim to ensure a stable dosing of antidepressant medication during the study.

To ensure that concomitant therapies are logged appropriately, subjects will be requested to report any changes to the researchers. Furthermore, concomitant therapies (pharmacotherapy, psychotherapy) will be logged at Screening, D1, D5 and follow-up visit. Subjects will be requested to include the dosing for these therapies (i.e., how often per day, how much in each pill, how many pills) as well as when they were first prescribed the medication.

6.5.1 RESCUE MEDICINE

N/A

7. STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTION

Discontinuation from the week of stimulation does not mean discontinuation from the study, and remaining study procedures should be completed as indicated by the study protocol. If a clinically significant finding is identified (including, but not limited to changes from baseline) after enrollment, the investigator or qualified designee will determine if any change in subject management is needed.

The study intervention (i.e., the 5 consecutive days of stimulation) will be discontinued for the following reasons:

- A subject develops significantly increased suicidal risk, as determined by an acute assessment by Dr. Schiller or Dr. Rubinow.
- A subject has a YMRS score greater than or equal to 12.

- The subject misses a single day of stimulation and is unable to make it up at the end of the stimulation week.
- The subject fails to complete the online suicidality questions within 72 hours of receiving the survey email and a personal phone contact does not explain the omission.
- Any clinical adverse event (AE), laboratory abnormality, intercurrent illness or other medical condition, or situation occurs such that continued participation in the study would not be in the best interest of the subject.
- The subject meets any exclusion criteria (either newly developed or not previously recognized).

If the subject withdraws from the intervention, the subject will be contacted and invited for the 2-week follow-up assessment including all respective questionnaires. This assessment is not intended as a clinical follow-up with the participants but serves research purpose only.

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Participants are free to withdraw from participation in the study at any time and without having to give a reason. But if the subject is willing to give a reason for discontinuation or withdrawal from the study it will be recorded with the subject files. Subjects who sign the informed consent form and are not randomized will be replaced. Subjects who sign the informed consent form, are randomized, and receive the full study intervention (5 consecutive days of 40-minutes of stimulation), and subsequently withdraw or are withdrawn or discontinue from the study will not be replaced. However, subjects who sign the informed consent form, and are randomized and receive only part of the study intervention, and subsequently withdraw or are withdrawn or discontinue from the study will be replaced.

7.3 LOST TO FOLLOW-UP

A participant will be considered lost to follow-up if he or she fails to return for the scheduled follow-up visit and is unable to be contacted by the study site staff. All efforts will be made to ensure subjects are not lost to follow-up, including developing rapport and ensuring enrolled subjects are reminded of their session dates. To ensure that subjects attend the follow-up session, research personnel will be flexible in timing, including offering sessions later in the day as well as some weekends.

Every effort will be made to contact subjects who are lost to follow-up, including contacting via email and phone. However, if a subject is lost to follow-up, the missed sessions will be labeled as missing data and our pre-determined analysis plan takes into consideration missing data.

8. STUDY ASSESSMENTS AND PROCEDURES

8.1 EFFICACY ASSESSMENTS

Inclusion and exclusion criteria will be determined at the initial two session (pre-screening and screening), including concomitant therapies, medical history, and diagnoses, to ensure that subjects are diagnosed with MDD, with low suicide risk, and free of benzodiazepines and anticonvulsant medications.

8.1.1 PHYSIOLOGICAL CHANGES

1. Resting state EEG (RSEEG) recordings will be completed several times during the study. On every day of the intervention week, subjects will complete an eyes open resting-state EEG (RSEEG) immediately before and after the 40-minutes of stimulation. This measure is used to determine the immediate after-effects of tACS on brain activity, specifically on alpha oscillation power, as well as how consecutive stimulation sessions may affect these after-effects. RSEEG will also be recorded at the follow-up visit to determine the lasting effects of stimulation. An eyes-closed RSEEG will also be collected on Day 1 and at the follow-up visit.

8.1.2 CLINICAL EVALUATIONS

1. The M.I.N.I is a diagnostic tool that will be used during the screening session to confirm diagnosis of unipolar, non-psychotic MDD.
2. The Hamilton Depression Rating Scale 17-item (HDRS17) [12] will be administered during the initial session, day 1 of stimulation, day 5 of stimulation and during the 2-week follow-up visit. This scale is used to determine eligibility and to monitor the severity of the subject's depressive symptoms, as well as determine suicide risk.
3. The C-SSRS is a standardized method to access lifetime suicidality and current suicidal ideations and intends.
4. The Maudsley Staging Method is a method of measuring treatment resistance in subjects with depression on a scale of 3 to 15 (mild = 3-6; moderate = 7-10; severe=11-15)[16].
5. The Young Mania Rating scale (YMRS) to detect symptoms of mania and hypomania during the study.

8.1.3 SELF-REPORT ASSESSMENTS

1. The Beck Depression Inventory (BDI) [17], [18]will be administered at day 1, day 5 of stimulation and at the follow-up visit. The BDI is a 21-item, self-report rating inventory that measures characteristic attitudes and symptoms of depression and will be used to monitor the severity of the patient's depression throughout the study.
2. The inventory of Depression and Anxiety Symptoms (IDAS) [19] will be completed day 1 of stimulation, day 5 of stimulation and during the 2-week follow-up visit. This inventory is used to monitor the perceived severity of the subject's symptoms of depression.
3. The Quality of Life Enjoyment and Satisfaction Questionnaire, short form (Q-LES-Q-SF)[20], will be administered at day 1 of stimulation, day 5 of stimulation, and the 2-week follow-up visit. This self-report questionnaire assesses quality of life.
4. The Pittsburg Sleep Quality Index (PSQI) [21], [22] will be administered at day 1 of stimulation and the 2-week follow up visit. This self-report questionnaire assesses sleep quality over the course of the past month. It will be used to determine changes in sleep quality.

5. The WHO Disability Assessment Schedule 2.0 (WHODAS 2.0) [23], [24] will be administered at baseline to assess any comorbid disability that could potentially affect quality of life and/or response to stimulation.
6. The Behavioral Inhibition and Behavioral Activation Self Report Scales (BAS/BIS) [25] will be completed during day 1 of stimulation, day 5 of stimulation and during the 2-week follow-up visit. These scales are used to monitor the perceived sensitivity to reward and punishment.
7. The State-Trait Anxiety Inventory (STAI) [26] will be completed during day 1 of stimulation, day 5 of stimulation and during the 2-week follow-up visit. These 40 items are used to monitor frequencies of feelings of anxiety.
8. The Snaith-Hamilton Pleasure Scale (SHAPS) [27] will be completed at day 1 of stimulation, day 5 of stimulation, and the 2-week follow-up visit. This scale is used to assess anhedonia.
9. The Beliefs about treatment questionnaires comprises four questions regarding participants expectations.
10. The Rumination scale assess recurring thought process on a 1-4 Likert Scale.
11. Blinding questionnaires for participant and operator are applied at D5.
12. Adverse Event questionnaires are administered after every stimulation session and ask about somatic symptoms/experiences during the tACS application.

8.2 SAFETY AND OTHER ASSESSMENTS

8.2.1 SUICIDAL IDEATIONS AND SUICIDAL BEHAVIOR

Only subjects with a HDRS-17 suicidality item score of <3 and low suicide risk based on the C-SSRS are enrolled in this study. Furthermore, suicidal thoughts and behaviors will be assessed using a brief daily suicide survey (IRB# 14-0600) consisting of three questions. Participants will be asked if they had any thoughts of hurting themselves in the last 24 hours (suicidal ideation, SI) and whether they have hurt themselves in the past 24 hours (suicidal behavior, SB). They will also be asked to rate the severity of their depression on a scale of 1 to 10. Participants receive a link to answer the daily suicide survey on RedCap. To maximize participant's safety the daily suicide survey is send during the follow-up period as well. Responses will be monitored by study personnel daily and the last survey will be administered at the day of follow-up visit. In addition, the C-SSRS [13] will be administered by trained research personnel at screening, D1, D5 and the follow-up session to thoroughly assess suicide risk.

If a participant report experiencing either SI or SB on either tool, research personnel will collect more information from the participant to deliver to either Dr. Schiller or Dr. Rubinow. Clinical personnel will decide if an acute assessment is required. Acute assessment may include facilitating contact of the subject with their psychiatrist or primary care physician to establish a plan for safety, continued care, and follow-up. If the participant does not have an established provider, Dr. Schiller or Dr. Rubinow will assist in establishing a care plan. If at any point during the assessment, the participant is deemed an imminent risk of harm to self or others, study personnel will enlist the aid of campus security to ensure that the participant is safely escorted to the Emergency Department for further care. Dr. Schiller and/or Dr.

Rubinow will decide if participation should be stopped after the acute assessment. If the participant is hospitalized, their participation will end.

8.2.2 DEVELOPMENT OF MANIA

The Young Mania Rating Scale (YMRS) [28] was designed to assess the severity of manic symptoms at enrolment, with the ability to track changes over the duration of participation. When undergoing treatment for depression, a possible side effect (as inferred from clinical trials with antidepressant medications) is to alter levels of serotonin, potentially associated with mania [29], [30]. Although we do not expect such an event to occur since we are not using a medication that targets serotonin levels, we will be conducting this assessment at Day 1, Day 5 and during the 2-week follow-up as a precautionary measure. If a participant develops any sign of mania (YMRS > 12), Dr. Schiller will conduct an acute assessment to determine if they are experiencing mania. If this occurs during the week of stimulation, participation in the study will be stopped and their primary mental health care provider or family physician will be contacted. In the case that they do not see anyone for their depression, Dr. Schiller will assist in creating a medical care plan

8.2.3 ADVERSE EVENT ASSESMENT

A structured interview probing for the experience of adverse events will be completed after each day of stimulation and at the follow up visit. Dr. Schiller or Dr. Rubinow will use the completed interview to assess the severity of the adverse event and its relationship to the study intervention as well determine if participation should be terminated.

8.2.4 STIMULATION RELATED SIDE EFFECT ASSESSMENT

A stimulation questionnaire will be administered at the end of each stimulation session. This tool will be used as a safety measure and to collect data on the participant's experience. A similar questionnaire was used in a previous study (IRB# 13-2995) to determine ability to successfully blind the participants using sham transcranial current stimulation.

8.3 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.3.1 DEFINITION OF ADVERSE EVENTS (AE)

Adverse event means any untoward medical occurrence associated with the use of an intervention in humans, whether considered intervention-related (21 CFR 312.32 (a)).

8.3.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

An adverse event (AE) or suspected adverse reaction is considered "serious" if, in the view of the investigator, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may

be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

8.3.3 CLASSIFICATION OF AN ADVERSE EVENT

8.3.3.1 SEVERITY OF EVENT

All adverse events (AEs) will be assessed by the principal investigator and/or co-investigator(s) using the following guidelines:

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious".

8.3.3.2 RELATIONSHIP TO STUDY INTERVENTION

All adverse events (AEs) must have their relationship to study intervention assessed by the principal investigator and co-investigator(s) who examines and evaluates the participant based on temporal relationship and their clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

- **Definitely Related** – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study intervention administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study intervention (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.
- **Probably Related** – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the study intervention, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.
- **Potentially Related** – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related", as appropriate.

- **Unlikely to be related** – A clinical event, including an abnormal laboratory test result, whose temporal relationship to study intervention administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the study intervention) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).
- **Not Related** – The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

8.3.3.3 EXPECTEDNESS

The principal investigator (Dr. Rubinow), with input from the co-investigator (Dr. Schiller) when necessary, will determine whether an adverse event (AE) is expected or unexpected in this population. The co-investigator (Dr. Flavio Frohlich) is an expert in non-invasive brain stimulation and will provide his expert opinion regarding this as well. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention.

8.3.4 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

The occurrence of an adverse event (AE) or serious adverse event (SAE) may come to the attention of study personnel during study visits, or the study subject may report AE or SAEs outside of a scheduled study visit. All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate case report form (CRF). Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution. Any medical condition that is present at the time that the subject is screened will be considered as baseline and not reported as an AE. However, if the study subject's condition deteriorates at any time during the study, it will be recorded as an AE. Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

Research personnel will record all reportable events with start dates occurring any time after informed consent is obtained until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation. At each study visit, research personnel will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization. All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate case report form (CRF). Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

8.3.5 ADVERSE EVENT REPORTING

We will be adopting the following reporting procedures:

What event is reported	When is event reported	By whom is event reported	To whom is event reported
Fatal or life-threatening unexpected, suspected serious adverse reactions	Within 24 hours of initial receipt of information	Investigator	• Local/internal IRB
Non-fatal, non-life-threatening unexpected, suspected serious adverse reactions	Within 48 hours of initial receipt of information	Research Personnel	• Local/internal IRB
Unanticipated adverse device effects	Within 10 working days of investigator first learning of effect	Investigator	• Local/internal IRB
Unanticipated Problem that is not an SAE	Within 7 days of the investigator becoming aware of the problem	Investigator	• Local/internal IRB

8.3.6 REPORTING OF PREGNANCY

Pregnancy tests to all women of child-bearing potential at the first laboratory visit, before the stimulation session. There is no evidence that suggests tACS would interfere with pregnancy [31]. However, should a subject become pregnant during the study their participation will be immediately terminated and they will be asked to consult with the Principal Investigator.

8.4 UNANTICIPATED PROBLEMS

8.4.1 DEFINITION OF UNANTICIPATED PROBLEMS (UP)

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to participants 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 Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;
- Related or possibly related to participation in the research ("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 participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

8.4.2 UNANTICIPATED PROBLEM REPORTING

If a UE occurs, the IRB will be notified, and the study will be adjusted as needed to protect the health and safety of the subjects. 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 subjects will be recruited and the research procedures for currently enrolled subjects will be stopped. Each UE will be recorded and reported throughout the study.

8.4.3 REPORTING UNANTICIPATED PROBLEMS TO PARTICIPANTS

Any new information gained during the study that may affect a participant's willingness to continue in the study will be reported to all currently enrolled subjects.

9. STATISTICAL CONSIDERATIONS

9.1 STATISTICAL HYPOTHESES

- Primary Efficacy hypothesis:
  - Null: There is no difference in changes of alpha frequency power between baseline RSEEG and RSEEG at completion of stimulation between treatment regimens.
  - Alternate: There is a difference in changes of alpha frequency power between baseline RSEEG and RSEEG at completion of stimulation between treatment regimens.

- Secondary Efficacy hypothesis:
  - Null: There is no relationship between the changes in alpha frequency power and changes in Hamilton Depression Rating Scale, 17 item (HDRS17) scores.
  - Alternate: There is a relationship between the changes in alpha frequency power and changes in Hamilton Depression Rating Scale, 17 item (HDRS17) scores.

9.2 SAMPLE SIZE DETERMINATION

In our recently concluded pilot clinical trial we compared the effects of 10 Hz tACS (therapeutic frequency) against 40 Hz tACS (control frequency) and sham/placebo stimulation. A total of 32 subjects enrolled, 30 completed the 5-day intervention, and 26 completed both a 2 and a 4-week follow-up session. With approximately 9 subjects in each group, a significant reduction was found in alpha oscillation power on the fifth day of stimulation ($p < 0.05$) [6]. Therefore, with a total of 20 subjects (10 in each group), we anticipate we will find a significant difference. Of note, our funding mechanism covers the cost of enrolling 20 participants.

9.3 POPULATIONS FOR ANALYSES

Every effort will be made to ensure all enrolled and randomized subjects complete all study sessions as described in this protocol. However, a priori, we determined that our population for analysis will be a modified intention-to-treat (ITT) analysis dataset. For this study, enrolled eligible subjects will be randomized to receive 5 consecutive days of stimulation. If a subject completes the intervention (i.e., receives all 5 consecutive days of stimulation), they will be included in all analyses moving forward.

As previously stated in ***Section 7 Study Intervention Discontinuation and Subject Discontinuation/Withdrawal***, enrolled subjects who do not complete the full intervention will be replaced. Therefore, with this population for analysis plan, we anticipate having data from 20 subjects that are eligible for analysis.

9.4 STATISTICAL ANALYSES

9.4.1 GENERAL APPROACH

All testing described below assumes a significance threshold of p = 0.05. Continuous data will be described using means and standard deviations, while categorical data will be described using counts/percentages.

There will be no pre-specified covariates described in this protocol. Data will be assessed for normality and, if deemed necessary, corrective procedures will be applied (e.g., log normalization).

9.4.2 ANALYSIS OF THE PRIMARY EFFICACY ENDPOINT(S) (EEG)

We will perform spectral analysis of resting state EEG from day 1 of stimulation (baseline), day 5 of stimulation, and the 2-week follow-up visit. We will use a repeated measure ANOVA and/or a general linear mixed-effects model (GLMM) to assess if there is statistically significant interaction between treatment (sham/placebo and 10 Hz tACS) and session (baseline, day 5 of stimulation, 2-week follow-up). In the case of missing data (e.g., missing follow-up visits), analysis using a GLMM will be prioritized, as GLMM takes into account missing data. Spectral analysis will be performed with multi-tapered estimation of the frequency spectrum followed by integration over the classical alpha EEG band (8-12 Hz).

In a separate analysis, we will perform spectral analysis of resting state EEG before and after each separate stimulation session. These analyses will be completed on the modified intention-to-treat dataset. Results from this section will be described in mean decibel (dB) change and presented using spectral images for visualization.

9.4.3 ANALYSIS OF THE SECONDARY ENDPOINT(S)(CORRELATION BETWEEN EEG CHANGES AND SYMPTOMS OF DEPRESSION)

Using the EEG results described in 9.4.2, mean dB change from baseline will be correlated with normalized change in HDRS17 scores.* Correlations will be assessed using Pearson's r and data will be plotted against the best fit line to visualize the results.

*HDRS17 normalization:
[[HDRS17 score at Visit #] - [HDRS17 score at baseline]]/[HDRS17 score at baseline]

9.4.4 SAFETY ANALYSES

As discussed in ***Section 8.2, Safety and Other Assessments*** safety will be assessed with the three questions from the daily suicide questionnaire, the C-SSRS, an adverse effects questionnaire, and the Young Mania Rating Scale (YMRS).

The suicide questionnaire is used as a screening to assess increase in suicidal ideation (SI) or suicidal behavior (SB) daily. The C-SSRS will be used to assess suicide risk more thoroughly. If a participant's responses on either tool indicate SI or SB, the subject will be contacted, and an acute assessment will be completed if deemed necessary by Dr. Schiller or Dr. Rubinow (as described above). Any verified increases in suicidal ideation will be described in counts/percentages and compared between groups using chi-square tests.

The stimulation questionnaire will be administered following every stimulation session, for a total of 5 administrations per participant. This questionnaire solicits ratings of 14 possible adverse effects associated with electrical stimulation, on a scale of 1 (absent) to 4 (severe). Paired t-tests with random effect "subject" will be calculated per adverse effect to determine if there are any differences in adverse effect severity between groups (sham/placebo, 10 Hz tACS). Severity per adverse effect will be described with mean and standard deviation.

The Young Mania Rating Scale (YMRS) will be used to assess any development of mania over the course of treatment. A YMRS score of greater than 12 indicates the possible development of a manic episode. Any subject who scores greater than 12 on the YMRS will be assessed by Dr. Schiller or Dr. Rubinow. Any verified manic episode will be described in counts/percentages and compared between groups using a chi-square test.

The Adverse Events Structured Interview will be administered by research personnel following every stimulation session and at the follow up visit, for a total of 6 time points per participant. This interview solicits open-ended descriptions of 11 possible adverse effects a participant may experience in any clinical trial as well as one question of ‘any other experiences’. Responses will be reviewed by the clinician and rated as described in ***Section 8.3.*** Paired t-tests will be calculated per adverse effect to determine if there are any differences in adverse effect severity between groups (sham/placebo, 10 Hz tACS). Severity per adverse effect will be described with mean and standard deviation.3.

9.4.5 BASELINE DESCRIPTIVE STATISTICS

All baseline descriptive statistics will be analyzed either using paired t-tests or chi-square tests when deemed appropriate. Descriptive statistics will be described based on the ***General Approach described in 9.4.1***.

9.4.6 PLANNED INTERIM ANALYSES

There are no other planned interim analyses.

9.4.7 SUB-GROUP ANALYSES

Sub-group analyses will not be used in this study, as the sample size is too small to conduct analyses based on age, sex, race/ethnicity, or other demographic characteristic(s).

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

10.1.1 INFORMED CONSENT PROCESS

10.1.1.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

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 and their families. Electronic consent and HIPAA consent will be obtained in the pre-screening remote session. The informed electronic consent will be documented via REDCap. A copy of the electronic consent and the HIPAA consent is handed out to the participant at the begin of D1. All consent forms will be IRB-approved and updated with any new information as modifications are made throughout the study.

10.1.1.2 CONSENT PROCEDURES AND DOCUMENTATION

At the begin of the remote pre-screening session, the researcher and potential participants will review the clinical trial in its entirety. At several intervals during the consent review, the researcher will ask questions that will assess the comprehension of the information in the consent. If the participant is unsure or does not know, the researcher will return to that section and more carefully explain the information. We will especially ensure that stimulation related side effects, the placebo arm of the study and the possibility to withdraw from the study at any given moment without prejudice are well understood. If needed, the participants will have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate.

At the beginning of the first visit to the lab (D1), participants will receive a paper copy of the electronic consent. The rights and welfare of the participant will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study

10.1.2 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform research staff, study subjects, and the IRB and will provide the reason(s) for the termination or suspension. Study subjects will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Increased suicide risk due to intervention (3 out of 8 participants or, if more than 8 enrolled, > 25% of participants require additional clinical care for their symptoms of depression)
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the IRB.

10.1.3 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the research team. This confidentiality is extended to cover the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. In addition, all research activities will be conducted in an as private as possible setting.

All data will only be referenced by dummy identifier code. Data will be stored on a password protected computer. A key connecting names and identifier 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 and there is no risk of deductive disclosure. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the IRB.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be entered into TraCS Clinical Research Data Management Service (REDCap). The database system provides secure web-based data entry with the data stored on servers that are maintained by TrACS. The data is encrypted during transmission. The servers are located in a secure campus area with all appropriate physical security measures in place. The web and database servers are monitored by the TraCS IT staff, patched frequently, and scanned by a third-party vendor to ensure that they are protected against known vulnerabilities. The scanning application is the standard service for the entire campus. Access is by individual user id and is restricted to the forms and/or functions that the user needs to have.

10.1.4 FUTURE USE OF STORED SPECIMENS AND DATA

Data collected for this study will be analyzed and stored within the Carolina Center for Neurostimulation/Frohlich Lab. After the study is completed, the data will be fully de-identified and archived within a locked file cabinet within the Carolina Center for Neurostimulation.

10.1.5 KEY ROLES AND STUDY GOVERNANCE

Provide the name and contact information of the Principal Investigator and the Medical Monitor.

Principal Investigator	**Co-Investigator**	**Co-Investigator**
David Rubinow, MD	Flavio Frohlich, PhD	Crystal Schiller, PhD

The University of North Carolina at Chapel Hill - Department of Psychiatry 919-445-0212 david_rubinow@med.unc.edu	The University of North Carolina at Chapel Hill - Department of Psychiatry 919-966-4584 Flavio_frohlich@med.unc.edu	The University of North Carolina at Chapel Hill - Department of Psychiatry 919-966-4810 crystal_schiller@med.unc.edu

10.1.6 SAFETY OVERSIGHT

Safety oversight will be under the direction of the Principal Investigator Dr. Rubinow. He will review all adverse events timely and serious adverse events and changes in the suicidality and mania ratings immediately. Based on his review, continuation of participant's participation is decided. All SAE or unanticipated AE will be reported to the local IRB.

10.1.7 CLINICAL MONITORING

The purpose of the monitoring plan is to present the approach of the Carolina Center for Neurostimulation to monitoring clinical trials. The plan facilitates compliance with good clinical practice.

(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.

10.1.7.1 THE CAROLINA CENTER FOR NEUROSTIMULATION MONITORING PLAN

The latest version of the approved IRB application for this clinical trial will be followed at all times. This responsibility falls in the hands of the trained research personnel. If at any time there is a deviation from protocol, the deviation form protocol log will be filled out. All team members will be trained on how and when to use this log.

Data will be verified for completeness following every study session and all data will be entered into REDCap, a secure online database. After a subject has completed their participation (full completion through the 2-week follow-up visit or because they withdrew prior to completion), data will be rereviewed for completeness and accuracy. After all data has been collected, data will be re-reviewed by another lab member who was not involved with the data collection process.

AE and SAE are clearly defined in the Master Protocol. Documents of AE and SAE can be found in the study binder on file within Dawson Hall Building, 77 Villcom Center Drive, Chapel Hill. It is responsibility of

trained research personnel to report all events to the PI. Reporting of AEs and SAEs is described within ***Section 8.3***.

The PI and Co-I will have read-only access to the REDCap database. This allows the PI and Co-I to view reports that provide information on any missing data on an individual subject basis, but does not allow them to add, change or input any data.

10.1.8 QUALITY ASSURANCE AND QUALITY CONTROL

The Carolina Center for Neurostimulation will conduct internal quality management of study conduct, data collection, documentation, and completion. Following written Standard Operating Procedures (SOPs), research personnel will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

10.1.9 DATA HANDLING AND RECORD KEEPING

10.1.9.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Trained research personnel will be responsible for the informed consent process, review for eligibility, questionnaire administration, data entry, device administration, EEG administration, and CRF entries. Research personnel 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.

Clinical data (including AEs, concomitant medications, and expected adverse reactions data) will be entered into a data capture system provided by TraCS Clinical Research Data Management Service (REDCap). The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents. Trained research personnel will have complete access to the REDCap system, while the PI and Co-I will have read-only ability. This will enable the researchers to enter the data and the PI and Co-I to review.

As discussed in ***Section 10.1.3*** , data entered into REDCap is stored on servers that are maintained by TrACS. The data is encrypted during transmission. The servers are located in a secure campus area with all appropriate physical security measures in place. The web and database servers are monitored by the TraCS IT staff, patched frequently, and scanned by a third party vendor to ensure that they are protected against known vulnerabilities. The scanning application is the standard service for the entire campus.

10.1.9.2 STUDY RECORDS 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.

10.1.10 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. The site PI/study staff will be responsible for knowing and adhering to their IRB requirements.

10.1.11 PUBLICATION AND DATA SHARING POLICY

This study will comply with the NIH Data Sharing Policy and Policy on the Dissemination of NIH-Funded Clinical Trial Information and the Clinical Trials Registration and Results Information Submission rule. As such, this trial will be registered at ClinicalTrials.gov, and results information from this trial will be submitted to ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-reviewed journals.

10.1.12 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence is critical. Any conflict of interest for any persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed by the UNC Conflict of Interest Office. If necessary, for persons who have a perceived conflict of interest, management will be provided again by the UNC Conflict of Interest office.

10.2 ADDITIONAL CONSIDERATIONS

N/A

10.3 ABBREVIATIONS

The list below includes abbreviations utilized in this template. However, this list should be customized for each protocol (i.e., abbreviations not used should be removed and new abbreviations used should be added to this list).

AE	Adverse Event
BDI	Beck Depression Inventory
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Amendments
CMP	Clinical Monitoring Plan
COC	Certificate of Confidentiality
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DSM-5	Diagnostic and Statistical Manual, 5th Edition
DSMB	Data Safety Monitoring Board
DRE	Disease-Related Event
EC	Ethics Committee
eCRF	Electronic Case Report Forms
EEG	Electroencephalogram
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
FFR	Federal Financial Report
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
GWAS	Genome-Wide Association Studies
HDRS	Hamilton Depression Rating Scale
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IDE	Investigational Device Exemption
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISM	Independent Safety Monitor
ISO	International Organization for Standardization
ITT	Intention-To-Treat
LSMEANS	Least-squares Means
MedDRA	Medical Dictionary for Regulatory Activities
MOP	Manual of Procedures
MSDS	Material Safety Data Sheet
NCT	National Clinical Trial
NIH	National Institutes of Health
NIH IC	NIH Institute or Center
OHRP	Office for Human Research Protections
PI	Principal Investigator
PSQI	Pittsburg Sleep Quality Index

Q-LES-Q-SF	Quality of Life Enjoyment & Satisfaction Questionnaire, Short Form
QA	Quality Assurance
QC	Quality Control
RSEEG	Resting state electroencephalogram
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SCID	Structured Clinical Interview for the DSM-5
SHAPS	Snaith-Hamilton Pleasure Scale
SMC	Safety Monitoring Committee
SOA	Schedule of Activities
SOC	System Organ Class
SOP	Standard Operating Procedure
UP	Unanticipated Problem
US	United States
YMRS	Young Mania Rating Scale
WHODAS 2.0	World Health Organization Disability Assessment Schedule 2.0

10.4 PROTOCOL AMENDMENT HISTORY

Version	Date	Description of Change	Brief Rationale
2	05/02/22	Update of several aspects	Increase Feasibility

11 REFERENCES

[1] I. H. Stanley, T. E. Joiner, and C. J. Bryan, "Mild traumatic brain injury and suicide risk among a clinical sample of deployed military personnel: Evidence for a serial mediation model of anger and depression," *J. Psychiatr. Res.*, vol. 84, pp. 161–168, 2017, doi: 10.1016/j.jpsychires.2016.10.004.

[2] A. J. Rush *et al.*, "Acute and longer-term outcomes in depressed outpatients requiring one or several treatment steps: a STAR*D report," *Am. J. Psychiatry*, vol. 163, no. 11, pp. 1905–1917, Nov. 2006, doi: 10.1176/ajp.2006.163.11.1905.

[3] A. R. Brunoni *et al.*, "Neuromodulation approaches for the treatment of major depression: challenges and recommendations from a working group meeting," *Arq. Neuropsiquiatr.*, vol. 68, no. 3, pp. 433–451, Jun. 2010, doi: 10.1590/s0004-282x2010000300021.

[4] J. B. Henriques and R. J. Davidson, "Regional brain electrical asymmetries discriminate between previously depressed and healthy control subjects," *J. Abnorm. Psychol.*, vol. 99, no. 1, pp. 22–31, Feb. 1990, doi: 10.1037//0021-843x.99.1.22.

[5] M. M. Ali, K. K. Sellers, and F. Fröhlich, "Transcranial alternating current stimulation modulates large-scale cortical network activity by network resonance," *J. Neurosci. Off. J. Soc. Neurosci.*, vol. 33, no. 27, pp. 11262–11275, Jul. 2013, doi: 10.1523/JNEUROSCI.5867-12.2013.

[6] M. L. Alexander *et al.*, "Double-blind, randomized pilot clinical trial targeting alpha oscillations with transcranial alternating current stimulation (tACS) for the treatment of major depressive disorder (MDD)," *Transl. Psychiatry*, vol. 9, no. 1, p. 106, 05 2019, doi: 10.1038/s41398-019-0439-0.

[7] T. Neuling, S. Rach, and C. S. Herrmann, "Orchestrating neuronal networks: sustained after-effects of transcranial alternating current stimulation depend upon brain states," *Front. Hum. Neurosci.*, vol. 7, p. 161, 2013, doi: 10.3389/fnhum.2013.00161.

[8] A. Vossen, J. Gross, and G. Thut, "Alpha Power Increase After Transcranial Alternating Current Stimulation at Alpha Frequency (α-tACS) Reflects Plastic Changes Rather Than Entrainment," *Brain Stimulat.*, vol. 8, no. 3, pp. 499–508, Jun. 2015, doi: 10.1016/j.brs.2014.12.004.

[9] F. H. Kasten, J. Dowsett, and C. S. Herrmann, "Sustained Aftereffect of α-tACS Lasts Up to 70 min after Stimulation," *Front. Hum. Neurosci.*, vol. 10, p. 245, 2016, doi: 10.3389/fnhum.2016.00245.

[10] A. Antal *et al.*, "Low intensity transcranial electric stimulation: Safety, ethical, legal regulatory and application guidelines," *Clin. Neurophysiol. Off. J. Int. Fed. Clin. Neurophysiol.*, vol. 128, no. 9, pp. 1774–1809, 2017, doi: 10.1016/j.clinph.2017.06.001.

[11] F. Fröhlich and D. A. McCormick, "Endogenous electric fields may guide neocortical network activity," *Neuron*, vol. 67, no. 1, pp. 129–143, Jul. 2010, doi: 10.1016/j.neuron.2010.06.005.

[12] J. B. Williams, "Standardizing the Hamilton Depression Rating Scale: past, present, and future," *Eur. Arch. Psychiatry Clin. Neurosci.*, vol. 251 Suppl 2, pp. II6-12, 2001, doi: 10.1007/BF03035120.

[13] K. Posner *et al.*, "The Columbia–Suicide Severity Rating Scale: Initial Validity and Internal Consistency Findings From Three Multisite Studies With Adolescents and Adults," *Am. J. Psychiatry*, vol. 168, no. 12, pp. 1266–1277, Dec. 2011, doi: 10.1176/appi.ajp.2011.10111704.

[14] A. R. Brunoni *et al.*, "The sertraline vs. electrical current therapy for treating depression clinical study: results from a factorial, randomized, controlled trial," *JAMA Psychiatry*, vol. 70, no. 4, pp. 383–391, Apr. 2013, doi: 10.1001/2013.jamapsychiatry.32.

[15] A. L. Stuart, J. A. Pasco, F. N. Jacka, S. L. Brennan, M. Berk, and L. J. Williams, "Comparison of self-report and structured clinical interview in the identification of depression," *Compr. Psychiatry*, vol. 55, no. 4, pp. 866–869, May 2014, doi: 10.1016/j.comppsych.2013.12.019.

[16] A. Fekadu, S. C. Wooderson, K. Markopoulou, and A. J. Cleare, "The Maudsley Staging Method for treatment-resistant depression: prediction of longer-term outcome and persistence of symptoms," *J. Clin. Psychiatry*, vol. 70, no. 7, pp. 952–957, Jul. 2009, doi: 10.4088/JCP.08m04728.

[17] A. T. Beck, R. A. Steer, and M. G. Carbin, "Psychometric properties of the Beck Depression Inventory: Twenty-five years of evaluation," *Clin. Psychol. Rev.*, vol. 8, no. 1, pp. 77–100, Jan. 1988, doi: 10.1016/0272-7358(88)90050-5.
[18] D. J. A. Dozois, K. S. Dobson, and J. L. Ahnberg, "A psychometric evaluation of the Beck Depression Inventory–II," *Psychol. Assess.*, vol. 10, no. 2, pp. 83–89, 1998, doi: 10.1037/1040-3590.10.2.83.
[19] D. Watson *et al.*, "Development and validation of the Inventory of Depression and Anxiety Symptoms (IDAS)," *Psychol. Assess.*, vol. 19, no. 3, pp. 253–268, Sep. 2007, doi: 10.1037/1040-3590.19.3.253.
[20] J. Endicott, J. Nee, W. Harrison, and R. Blumenthal, "Quality of Life Enjoyment and Satisfaction Questionnaire: a new measure," *Psychopharmacol. Bull.*, vol. 29, no. 2, pp. 321–326, 1993.
[21] D. J. Buysse, C. F. Reynolds, T. H. Monk, S. R. Berman, and D. J. Kupfer, "The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research," *Psychiatry Res.*, vol. 28, no. 2, pp. 193–213, May 1989, doi: 10.1016/0165-1781(89)90047-4.
[22] J. S. Carpenter and M. A. Andrykowski, "Psychometric evaluation of the Pittsburgh Sleep Quality Index," *J. Psychosom. Res.*, vol. 45, no. 1, pp. 5–13, Jul. 1998, doi: 10.1016/s0022-3999(97)00298-5.
[23] G. Andrews, A. Kemp, M. Sunderland, M. Von Korff, and T. B. Ustun, "Normative Data for the 12 Item WHO Disability Assessment Schedule 2.0," *PLoS ONE*, vol. 4, no. 12, Dec. 2009, doi: 10.1371/journal.pone.0008343.
[24] J. Rehm *et al.*, "On the development and psychometric testing of the WHO screening instrument to assess disablement in the general population," *Int. J. Methods Psychiatr. Res.*, vol. 8, no. 2, pp. 110–122, 1999, doi: 10.1002/mpr.61.
[25] C. S. Carver and T. L. White, "Behavioral inhibition, behavioral activation, and affective responses to impending reward and punishment: The BIS/BAS Scales," *J. Pers. Soc. Psychol.*, vol. 67, no. 2, pp. 319–333, 1994, doi: 10.1037/0022-3514.67.2.319.
[26] L. J. JULIAN, "Measures of Anxiety," *Arthritis Care Res.*, vol. 63, no. 0 11, Nov. 2011, doi: 10.1002/acr.20561.
[27] R. P. Snaith, M. Hamilton, S. Morley, A. Humayan, D. Hargreaves, and P. Trigwell, "A scale for the assessment of hedonic tone the Snaith-Hamilton Pleasure Scale," *Br. J. Psychiatry J. Ment. Sci.*, vol. 167, no. 1, pp. 99–103, Jul. 1995, doi: 10.1192/bjp.167.1.99.
[28] R. C. Young, J. T. Biggs, V. E. Ziegler, and D. A. Meyer, "A rating scale for mania: reliability, validity and sensitivity," *Br. J. Psychiatry J. Ment. Sci.*, vol. 133, pp. 429–435, Nov. 1978, doi: 10.1192/bjp.133.5.429.
[29] E. Y. Kim *et al.*, "Rate of diagnostic conversion to bipolar disorder in adults with unipolar depression and psychopharmacological treatment in the republic of Korea: A nationwide register-based study," *J. Affect. Disord.*, vol. 273, pp. 240–246, Aug. 2020, doi: 10.1016/j.jad.2020.03.159.
[30] L. M. Melhuish Beaupre *et al.*, "Antidepressant-Associated Mania in Bipolar Disorder: A Review and Meta-analysis of Potential Clinical and Genetic Risk Factors," *J. Clin. Psychopharmacol.*, vol. 40, no. 2, pp. 180–185, Apr. 2020, doi: 10.1097/JCP.0000000000001186.
[31] G. N. Konstantinou, S. N. Vigod, S. Mehta, Z. J. Daskalakis, and D. M. Blumberger, "A systematic review of non-invasive neurostimulation for the treatment of depression during pregnancy," *J. Affect. Disord.*, vol. 272, pp. 259–268, Jul. 2020, doi: 10.1016/j.jad.2020.03.151.