Closed-Loop Transcranial Alternating Current Stimulation for the Treatment of Depression (CLACS): Single-Site Open-Label Pilot Study

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Summary of Changes from Previous Version:

Protocol Date	Summary of Revisions Made
30Jan2023	Original protocol
05Jun2023	Addition of self-scoring surveys up to 12-week post treatment follow-up; Addition of EEG at Day 1/ Baseline and at the 2-week post treatment follow-up
28Nov2023	2-week post treatment follow up visit must be done in person (no remote option)
21Dec2023	Enrollment increase from 20 to 35 participants
16Feb2024	Addition of HDRS-17 assessment at the 6-week post treatment follow-up

NIH-FDA Phase 2 and 3 IND/IDE Clinical Trial Protocol Template

1. 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)

All members of the study team have completed Human Participants Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant 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 participant 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 participants who provided consent, using a previously approved consent form.

2. PROTOCOL SUMMARY

2.1 Schedule Of Activities (SOA)

Procedures	Electronic Prescreening Survey	Remote Screening (D0) Remote/in-person	Day 1 of Stimulation (Baseline/D1) In-person	Day 2 of Stimulation (D2) In-person	Day 3 of Stimulation (D3) In-person	Day 4 of Stimulation (D4) In-person	Day 5 of Stimulation (D5) In-person	1-week follow-up FU 1 (D12) Remote	2-week follow-up FU 2 (D19) in-person	4-week follow-up FU 3 (D33) Remote	6-week follow-up FU 4 (D47) in-person	8-week follow-up FU 5 (D61) Remote	10-week follow-up FU 6 (D75) Remote	12-week follow-up FU 7 (D89) Remote
Waived/ Verbal Consent	X	X												
Contact information, MDD diagnosis, exclusion/inclusion criteria	X													
Informed consent			X											
Medication & psychotherapy			X				X		X	X	X	X	X	X
Determine Eligibility		X												
Demographics		X												
Medical history		X												
Maudsley Staging Method		X												
M.I.N.I.		X												
C-SSRS Triage			X						X		X			
HDRS-17		X	X				X		X		X			

Procedures	Electronic Prescreening Survey	Remote Screening (D0) Remote/in-person	Day 1 of Stimulation (Baseline/D1) In-person	Day 2 of Stimulation (D2) In-person	Day 3 of Stimulation (D3) In-person	Day 4 of Stimulation (D4) In-person	Day 5 of Stimulation (D5) In-person	1-week follow-up FU 1 (D12) Remote	2-week follow-up FU 2 (D19) in-person	4-week follow-up FU 3 (D33) Remote	6-week follow-up FU 4 (D47) in-person	8-week follow-up FU 5 (D61) Remote	10-week follow-up FU 6 (D75) Remote	12-week follow-up FU 7 (D89) Remote
QIDS (S)			X				X	X	X	X	X	X	X	X
ASRM (S)		X	X				X	X	X	X	X	X	X	X
SHAPS (S)			X				X	X	X	X	X	X	X	X
DASS-42 (S)			X				X	X	X	X	X	X	X	X
STAI (trait) (S)			X											
STAI (state) (S)			X				X	X	X	X	X	X	X	X
Q-LES-Q-SF (S)			X				X	X	X	X	X	X	X	X
CGI			X				X		X					
CLtACS			X	X	X	X	X							
CLtACS Side Effect Questionnaire			X	X	X	X	X							
Likert State Scale (Mood)			X	X	X	X	X							
(HD)-EEG			X[1]						X[2]					
AE Structured Interview							X							
Treatment Experience Questionnaire							X							

(S) = RedCap online survey; ASRM: Altman Self-Rating Mania Scale; SHAPS: Snaith-Hamilton Pleasure Scale; DASS-42: Depression Anxiety and Stress Scale; QIDS: Quick Inventory of Depressive Symptomatology; STAI: State-Trait Anxiety Inventory; CGI: Clinical Global Impression Scale; 1. HD-EEG is performed pre-stimulation and post-stimulation; 2. Visit must be done in person for participants undergoing the EEG assessment

2.2 SYNOPSIS

Title:	Closed-Loop Transcranial Alternating Current Stimulation (CLtACS) for the Treatment of Depression: Open-label Pilot Study
Study Description:	The purpose of this clinical trial is to investigate the preliminary efficacy of CLtACS for the treatment of major depressive disorder (MDD) in an open-label pilot study. We will recruit up to 35 participants with unipolar, non-psychotic MDD. Eligible participants will have five up to 40-minute stimulation sessions over five consecutive days. All participants will receive CLtACS at their individual peak alpha frequency. Participation will include 13 visits, six of them remotely (with an in-person option as needed), and one electronic survey. Potential participants fill-in an electronic pre-screening form. If preliminarily eligible, a remote screening visit is performed. If eligible, participants attend five consecutive, daily stimulation sessions. Clinical assessments will be performed at Baseline/ day 1 of stimulation (D1), day 5 of stimulation (D5), at follow-up visit 2 (FU2)/ 2 weeks after completion of stimulation (D19), and at follow-up 4 (FU4)/ 6 weeks after completion of stimulation (D47) using the Hamilton Depression Rating Scale (HDRS-17). 7 Self-scoring surveys will be sent after completion of stimulation (FU1, FU2, FU3/ 4 weeks after completion of stimulation, FU4, FU5/ 8 weeks after completion of stimulation, FU6/ 10 weeks after completion of stimulation, FU7/ 12 weeks after completion of stimulation) until 12 weeks after completion of stimulation (D89). Eyes-open and eyes-closed resting state recordings will be collected before and after CLtACS on D1 and during the FU2 visit to measure changes in alpha oscillations.
Objectives:	Primary Objective: To investigate the preliminary efficacy in reducing depression symptom severity by CLtACS in people with MDD over the course of a 5-day, 40-minute stimulation protocol. Secondary Objective: To investigate the preliminary efficacy in terms of response and remission rates. To investigate the neurophysiological effects of CLtACS on brain activity by means of HD-EEG.

Endpoints:	Primary Endpoint: Change in HDRS-17 between two-week follow-up (FU2) and Day 1 (D1). Secondary Endpoints: ● Change in HDRS-17 between D5 and Baseline/D1. ● Response and remission rates of depression at D5, FU2 ● Change in Quick Inventory of Depressive Symptomatology (QIDS) at D5, FU1, FU2 ● Change in Altman Self-Rating Mania Scale (ASRM) at D5, FU1, FU2 ● Change in Snaith-Hamilton Pleasure Scale (SHAPS) at D5, FU1, FU2 ● Change in Depression Anxiety and Stress Scale (DASS-42) at D5, FU1, FU2 ● Change in State-Train Anxiety Inventory (STAI) at D5, FU1, FU2 ● Change in Quality-of-Life Enjoyment and Satisfaction Questionnaire, short form (Q-LES-Q-SF) at D5, FU1, FU2 ● Change in Clinical Global Impression Scale (CGI) at D5 and FU2 Exploratory Endpoints: ● Feasibility - fraction of participants who complete the 5-day course of stimulation ● Change in HD-EEG alpha oscillation power at D1 pre-stimulation, D1 post-stimulation, and FU2 ● Change in Quick Inventory of Depressive Symptomatology (QUIDS) at FU3, FU4, FU5, FU6, FU7 ● Change in Altman Self-Rating Mania Scale (ASRM) at FU3, FU4, FU5, FU6, and FU7 ● Change in Depression Anxiety and Stress Scale (DASS-42) at FU3, FU4, FU5, FU6, and FU7 ● Change in State-Train Anxiety Inventory (STAI) at FU3, FU4, FU5, FU6, and FU7 ● Change in Snaith-Hamilton Pleasure Scale (SHAPS) FU3, FU4, FU5, FU6, and FU7 ● Change Quality of Life Enjoyment and Satisfaction Questionnaire, short form (Q-LES-Q-SF) at FU3, FU4, FU5, FU6, and FU7 ● Change in HDRS-17 between FU4 and D1
Study Population:	We will recruit up to 35 participants (age 18 and older) with a diagnosis of unipolar, non-psychotic MDD. Eligible participants will have a HDRS-17 > 8 and low suicide risk (no active intent as determined by the Columbia Suicide Severity Rating Scale, C-SSRS, triage form). Participants will be recruited from the Chapel Hill, Durham, and Raleigh area*s.*
Phase:	N/A
Description of Sites/Facilities Enrolling Participants:	University of North Carolina at Chapel Hill

Description of Study Intervention:	We will use the XCSITE100Pro stimulator designed by Pulvinar Neuro for investigational purposes to deliver closed-loop transcranial alternating current stimulation (CLtACS). Stimulation waveforms are sine-waves with a zero-to-peak amplitude of 2mA delivered to F3 and F4 with a return electrode at Cz. Participants will stay in a relaxed yet experimentally controlled state by watching a nature movie such as “Reefscape'' during stimulation.
Study Duration:	12 months
Participant Duration:	Participation for each participant will last no longer than 13 weeks total. We estimate total participation time to be approximately 13 hours.

3. INTRODUCTION

3.1 Study Rationale

Major depressive disorder (MDD) is a common, often 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 not effective for all people with MDD and can result in undesirable side effects. Prominently, 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.

3.2 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].
Preclinical and computational studies have contributed to a growing understanding of the mechanism of action of tACS [7]. At the center of successful target engagement is the modulation of neuronal network oscillations by carefully tuned stimulation waveforms that match the endogenous dynamics. Given the low amplitude of the electric field delivered to the brain, modulation of oscillations requires synergistic interaction between the rhythmic stimulation waveform and endogenous brain rhythms *[8]*. Closed-loop stimulation allows the adjustment of the stimulation to the fluctuations in individual brain network activity patterns and thus is poised to enhance the efficacy in terms of modulating the targeted oscillation. Briefly, closed-loop stimulation combines periodic measurement of brain activity patterns with EEG and stimulation that is tuned based on the dynamic properties of the measured brain activity. Measurement and stimulation are interleaved due to the electric artefacts caused by stimulation. This approach has been pioneered by the Frohlich Lab and was demonstrated to successfully modulate another, transient thalamo-cortical activity pattern, sleep spindles and associated memory consolidation *[9]*. Closed-loop stimulation is thus expected to ultimately increase efficacy through improved targeting *[10], [11]*. Alternative strategies would include increasing the cumulative dose of the stimulation, which would be more burdensome for the participants (longer and or more sessions). Given the promising clinical results of the previous 5-day paradigm, increasing the cumulative dose is not a priority. In

addition, stimulation could be combined with other therapeutic interventions such as therapy. Examining such synergistic effects are outside the scope of this study.
This current study aims to improve efficacy by delivering targeted, individualized stimulation based on closed-loop tACS for which the stimulation waveform is adjusted based on interleaved EEG measurements.

3.3 Risk/Benefit Assessment

3.3.1 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. 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 participant dummy identifiers, or stored in pass-word protected spreadsheets only accessible to study personnel. All data is stored in locked cabinets inside locked offices; electronic data will be stored only on password-protected computers. Interviews will be conducted over the HIPAA approved Zoom account at UNC. Only study personnel will have access to the data. All study staff participate in annual human participant 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, which may lead to emotions of embarrassment. This risk is necessary in order to assess mood symptoms and associated psychopathology. Participants 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 [12]. 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 [13]. However, tACS does have some mild side effects, such as transient mild tingling, burning, or itching under the electrode sites. [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 are present during the full experiment.

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. Rather, active engagement in a clinical trial may offer hope and structure to the participants. Regardless, participants with high suicide risk (intent or plan) will not be included in this study. If an enrolled participant 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 Columbia Suicide Severity Rating Scale (C-SSRS, triage form) [14] to determine intent for suicide during D1. Inclusion criteria state that the participant must be low suicide risk and that

potential participants with an above "low risk" designation will not be eligible for the study. In the event that suicide risk increases during participation in the study, the participant will be asked to stop the study. The participant will be provided with a referral to UNC Department of Psychiatry, and their mental health care or family medical doctor will be contacted.

3.3.2 Known Potential Benefits

This study has not been designed to benefit the individual participants.

3.3.3 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. Furthermore, this is an open-label study that does not require considerations about the risks associated with a placebo group. We also note that the FDA has recently confirmed a non-significant risk (NSR) status for a very similar tACS trial in MDD.

4. OBJECTIVES AND OUTCOME MEASURES

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
To determine the effect of CLtACS on depression symptom severity	Change in HDRS-17 between two-week follow-up (FU2) and Day 1 (D1)	The HDRS-17 is the standard clinician-administered instrument to measure change in symptom severity in people with MDD
Secondary		
To determine the effect of CLtACS on depression symptom severity	Change in HDRS-17 between D5 and Baseline/ D1	The HDRS-17 is the standard clinician-administered instrument to measure change in symptom severity in people with MDD.
To determine the remission and response rate of CLtACS Response is defined as >= 50% reduction rate in HDRS-17 score relative to D1 Remission is defined as HDRS-17 < 8	Number of response/remission rates at D5 and FU2	Remission and response rates provide additional insights into the efficacy of a treatment.

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
To determine time-course of symptom change	Change in Quick Inventory of Depressive Symptomatology (QIDS) at D5, FU1, FU2	Battery of self-report measures of mood and related constructs at the 7-day follow up (digitally delivered) provide additional insights into the symptom changes in between the clinician administered assessment on Day 5 and at FU2.
	Change in Altman Self-Rating Mania Scale (ASRM) at D5, FU1, FU2	
	Change in Snaith-Hamilton Pleasure Scale (SHAPS) at D5, FU1, FU2	
	Change in Depression Anxiety and Stress Scale (DASS-42) at D5, FU1, FU2	
	Change in State-Train Anxiety Inventory (STAI) at D5, FU1, FU2	
	Change in Quality of Life Enjoyment and Satisfaction Questionnaire, short form (Q-LES-Q-SF) at D5, FU1, FU2	
	Change in Clinical Global Impression Scale (CGI) at D5, FU2	
Exploratory		
Feasibility	Fraction of participants who complete the 5-day course of stimulation	Since the primary outcome is determined from the subgroup of people who have completed stimulation, examining the fraction of people who complete the stimulation paradigm is important to minimize bias in case of people discontinuing study participation due to lack of perceived efficacy or symptoms worsening.
To determine the effect of CLtACS on endogenous EEG alpha oscillations	Change in HD-EEG alpha oscillation power at D1 pre-stimulation, D1 post-stimulation, and FU2	The inclusion of EEG endpoints provides insights into the network-level mechanisms underlying the treatment effects.

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
To determine time-course of symptom change	Change in Quick Inventory of Depressive Symptomatology (QUIDS) at FU3, FU4, FU5, FU6, FU7	Battery of self-report measures of mood and related constructs provide additional insights into the symptom changes in between the clinician administered assessment on D5, FU2, and FU4. Time-scale of symptom change will provide useful information for the design of future, large-scale treatment studies.
	Change in Altman Self-Rating Mania Scale (ASRM) at FU3, FU4, FU5, FU6, and FU7	
	Change in Depression Anxiety and Stress Scale (DASS-42) at FU3, FU4, FU5, FU6, and FU7	
	Change in State-Train Anxiety Inventory (STAI) at FU3, FU4, FU5, FU6, and FU7	
	Change in Snaith-Hamilton Pleasure Scale (SHAPS) FU3, FU4, FU5, FU6, and FU7	
	Change Quality of Life Enjoyment and Satisfaction Questionnaire, short form (Q-LES-Q-SF) at FU3, FU4, FU5, FU6, and FU7	
To determine the effect of CLtACS on depression symptom severity	Change in HDRS-17 between six week follow up (FU4) and D1	The HDRS-17 is the standard clinician-administered instrument to measure change in symptom severity in people with MDD.

5. STUDY DESIGN

5.1 Overall Design

The design for this study is a pilot, open-label clinical trial which will be used to investigate the preliminary efficacy of closed-loop alpha-tACS for the treatment of depression. We are recruiting from a clinical population. Participants will be up to 35 people ages 18-70 with unipolar, non-psychotic MDD, who have a HDRS-17 > 8 and are at a low risk for suicide according to the Columbia Suicide Severity Rating Scale (C-SSRS). Nursing and pregnant participants 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 or receive concurrent mental health care from a family practitioner, therapist, or psychiatrist.

This is a single-site, pilot clinical trial with a single arm. We will use the XCSITE100Pro stimulator designed by Pulvinar Neuro for investigational purposes to deliver closed-loop stimulation over five consecutive days. Participants 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 2 hours. D2-D4 will take 1 hour. The 2-week (FU2) and 6-week (FU4) follow-up will take

approximately 1 hour. Completion of patient-administered surveys at FU1 to FU7 will take about 30 minutes to complete. We estimate that total participation to be approximately 13 hours.

5.2 Scientific Rationale for Study Design

This study is an open-label pilot interventional study. Given the novelty of the treatment and the absence of data to estimate effect sizes, an initial open-label trial is warranted.

5.3 Justification for Dose

We are utilizing the same dosage as given in a similar pilot study: 5 consecutive days of up to 40-minutes of stimulation [6].

5.4 End of Study Definition

The end of this study is defined as when the last participant completes the final study visit (i.e., the last participant completes the FU7 study visit).

6. STUDY POPULATION

6.1 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-V diagnosis of MDD; unipolar, non-psychotic
- Hamilton Rating Depression Rating Scale (HRDS-17) score >8
- Low suicide risk as determined by the C-SSRS triage form (no intent or plan)
- Capacity to understand all relevant risks and potential benefits of the study (informed consent)

6.2 Exclusion Criteria

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

- DSM-5 diagnosis of severe alcohol use disorder (AUD) within the last 12 months
- DSM-5 diagnosis of moderate to severe substance use disorder (excluding tobacco) within the last 12 months
- Lifetime history of bipolar disorder, psychotic disorder, schizophrenia, autism
- Current use of benzodiazepines > 20mg diazepam/d equivalent
- Antidepressant dose change within the last 2 weeks
- Initiated new antidepressant within the last 4 weeks
- Initiated psychotherapy within the last 4 weeks
- Initiated other form of non-medication, non-neurostimulation treatment within the last 4 weeks
- Currently or history (3 months) of receiving TMS, ketamine, esketamine, or ECT.
- Previously failed to respond to ECT or TMS
- History of seizure (excluding febrile seizures in childhood or ECT induced seizures)

- Neurological disorders that would increase risk of participation or present a significant confounder in the opinion of the investigator (for example, dementia, history of stroke, Parkinson's disease, multiple sclerosis, history of traumatic brain injury with prolonged loss of consciousness, ruptured cerebral aneurysm, previous CNS radiation)
- Unstable medical disorder in the eye of the study physician
- Prior brain surgery and/or brain implants
- Current pregnancy or breastfeeding
- Unwillingness to use appropriate birth control if sexually active
- Currently enrolled in another clinical trial for depression
- 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

6.3 Lifestyle Considerations

People of child-bearing potential who are sexually active are asked to use an appropriate method of birth control.

6.4 Screen Failures

In the case that a participant does not qualify based on suicide risk, established procedures in the Carolina Center for Neurostimulation will be followed to ensure participant safety.

6.5 Strategies for Recruitment and Retention

This clinical trial will utilize multiple recruitment strategies to communicate this opportunity to as many potential participants as possible. 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. 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, 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. We will also use radio spots, newspaper ads, billboards, and in-person recruitment as needed. 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.

[1]

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 participant. The participant will receive payment in the form of a Visa Card at D1 ($100). This card will be charged again on D5 ($100), and the final follow-up session ($100). Thus, completion of the study will result in a financial compensation of $300.

Research personnel will be easily available for the participants to contact via email or phone. The inclusion criteria state that each participant must be able to understand all risks and benefits associated with this study. We will be asking each participant to answer questions about the consent form to determine that the study process and the duration of participation are completely understood by all participants. We will aim to have a specific research team member assigned to complete all sessions with the same participant. 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 participant so they feel comfortable and willing to discuss what may be sensitive information. Retention will be quantified by the fraction of participants 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 participants 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 participant will be withdrawn from participation. This schedule would permit the participant 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).

7. STUDY INTERVENTION

7.1 Study Intervention(s) Administration

7.1.1 Study Intervention Description

We will be using the XCSITE100Pro stimulator designed by Pulvinar Neuro. 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 participant. There has never been an instance of serious side-effect reported due to the use of transcranial alternating current stimulation (tACS).

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 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 pre-programmed for closed-loop, and is not designed for tACS clinical trials. This makes the use of the Neuroconn device not appropriate for this study. The XCSITE 100 Pro includes identical stimulation hardware, including the safety features listed below, as the XCSITE 100.

The XSCITE100Pro 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 8 mA (peak-to-peak for tACS). For the purposes of this study, this device will be set to deliver closed-loop tACS in the alpha frequency with a peak amplitude of 2 mA. For more instructions, please see the submitted instruction manual.

The stimulator includes the following safety features:

Current Sensor Circuit. A 33.2 Ω sense resistor is placed in series with the stimulation electrodes on the high side. Since high-side current sensing is used, any short circuit of the electrode terminals to ground will be detected. The stimulation current flows through this resistor and creates a voltage. The voltage across this resistor is sensed and amplified by the AD628 difference amplifier.

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 ±4 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 ± 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 triggered. This fuse is sized with a cutoff of 200% of steady-state operating current.

7.1.2 Dosing and Administration

The research team will first measure each participant's head using the 10-20 system to determine the electrode locations. Participants will then be fitted with the 3 electrodes for stimulation: two 5x5cm electrodes placed over F3 (left frontal cortex, standard 10-20 EEG coordinated) and F4 (right frontal cortex), and one 5x7cm electrode placed over Cz (midline, central). Electrodes will be carbon rubber, with Ten20 conductive paste applied. During stimulation, the participant 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 up to 40-minutes. Before each stimulation session, a single-channel eyes open and eyes closed resting state EEG will be performed to individualize the treatment frequency.

The stimulation waveforms used in this study are tACS at the individual peak alpha frequency at 1 mA zero-to-peak amplitude both at F3 and F4, achieved via a splitter cable to distribute 2 mA between the two sites. Stimulation includes a ramp-in and ramp-out of 20 seconds, with a maximum of 40-minutes of stimulation at an amplitude of 1 mA per electrode, for a total of 2440 seconds of stimulation.

Research personnel will be thoroughly trained and have training 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. At D5, a systematic interview for side-effect is administered.

7.2 Measures to Minimize Bias: Randomization and Blinding

Randomization
There is no randomization since this is an open-label pilot study.

Blinding
There is no blinding since this is an open-label pilot study.

7.3 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.

7.4 Concomitant Therapy

Eligible participants will be permitted to be receiving concomitant therapy, such as psychotherapy, antidepressants, or other medications. However, eligibility for this trial includes a stable antidepressant medication dose for the past 2 weeks before the screening visits. Moreover, a new antidepressant must not be started during the last 4 weeks before the screening visit. Participants who are not taking medication for the treatment of MDD are also included.

To ensure that concomitant therapies are logged appropriately, participants 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. Participants 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.

8. STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

8.1 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 participant management is needed.

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

- A participant develops significantly increased suicidal risk, as determined by an acute assessment by Dr. Schiller or Dr. Rubinow.
- A participant has an ASRM score greater than or equal to 6.
- The participant misses a single day of stimulation and is unable to make it up at the end of the stimulation week.
- 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 participant.
- The participant meets any exclusion criteria (either newly developed or not previously recognized).

If the participant withdraws from the intervention, the participant will be contacted and invited for the FU1 survey questionnaires and for the FU2 follow-up assessment including all respective questionnaires. These assessments are not intended as a clinical follow-up with the participants but serve research purposes only.

8.2 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. If the participant is willing to give a reason for discontinuation or withdrawal from the study, it will be recorded with the participant files. Participants who sign the informed consent form and do not receive stimulation will be replaced. Participants who sign the informed consent form and receive only part of the study intervention OR withdraw or are withdrawn or discontinue from the study prior to the 2-week follow-up will not be replaced. The maximum number of participants consented will be 35.

8.3 Lost to Follow-Up

A participant will be considered lost to follow-up if they fail to return for the scheduled follow-up visit and are unable to be contacted by the study site staff. All efforts will be made to ensure participants are not lost to follow-up, including developing rapport and ensuring enrolled participants are reminded of their session dates. To ensure that participants 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 participants who are lost to follow-up, including contacting via email and phone. However, if a participant 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.

9. STUDY ASSESSMENTS AND PROCEDURES

9.1 Efficacy Assessments

Inclusion and exclusion criteria will be determined by the electronic pre-screening form and the virtual screening visit, including documentation of concomitant therapies, medical history, and diagnoses, to ensure that participants are diagnosed with MDD, and with low suicide risk.

9.1.1 Clinical Evaluations

1. The M.I.N.I is a diagnostic tool that will be used during the screening session to confirm diagnosis of MDD.
2. The Hamilton Depression Rating Scale 17-item (HDRS17) [15] is a scale is used to determine eligibility and to monitor the severity of the participant's depressive symptoms.
3. The C-SSRS (triage form) is a standardized method to assess suicide risk.
4. The Maudsley Staging Method is a method of measuring treatment resistance in participants with depression on a scale of 3 to 15 (mild = 3-6; moderate = 7-10; severe=11-15) [16].
5. The Altman Self-Rating Mania Scale (ASRM) is a method to detect symptoms of mania and hypomania during the study.

9.1.2 Self-Report Assessments

1. The Quick Inventory of Depressive Symptomatology (QIDS) is a 16-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 Quality of Life Enjoyment and Satisfaction Questionnaire, short form (Q-LES-Q-SF)[17] is a self-report questionnaire that assesses quality of life.
3. Stimulation side-effect questionnaires are administered after every stimulation session. Systematic query of side effects across all major organ systems is administered at D5.

9.1.3 High-Density (HD) EEG

Eyes-open and eyes-closed resting state recordings will be collected before and after CL-tACS on D1 and during the FU2 visit to measure changes in alpha oscillations. High-Density (HD)-EEG will be performed using a HydroCel Geodesic Sensor Net (128 channel) with a Net Amps 400 series amplifier (Magstim EGI, Eugene, OR, USA) with whole scalp coverage.

9.2 Safety and Other Assessments

9.2.1 Suicidal Ideations And Suicidal Behavior

Only participants deemed at low suicide risk based on the C-SSRS (ideation allowed, however intent, plan or attempt excluded) are enrolled in this study. The C-SSRS [14] will be administered by trained research personnel at screening and the follow-up session to thoroughly assess suicide risk. In addition, the answers to suicide-related questions on the HRDS-17 and on the QIDS will be monitored. Based on these sources of information, clinical personnel will decide if an acute assessment is required. Acute assessment may include facilitating contact of the participant 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 security at the facility or first responders 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.

9.2.2 Development Of Mania

The Altman Self-Rating Mania Scale (ASRM) 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 [18], [19]. Although we do not expect such an event to occur since there are no reports of inducing mania by tACS and we are not using a medication that targets serotonin levels, we will be conducting this assessment as a precautionary measure. If a participant develops any sign of mania (ASRM > 6), Dr. Schiller or Dr. Rubinow will conduct an acute assessment to determine if the participant is 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 mental health concerns, Dr. Schiller or Dr. Rubinow will assist in creating a medical care plan.

9.2.3 Adverse Event Assessment

A self-report form of sensory experiences associated with stimulation and side-effects will be administered at D1-D5. In addition, a structured interview probing for the experience of adverse events will be administered at D5. Dr. Schiller or Dr. Rubinow will use the completed interview to assess the severity of the adverse event and its potential relationship to the study intervention.

9.3 Adverse Events and Serious Adverse Events

9.3.1 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)).

9.3.2 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 participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

9.3.3 Classification of an Adverse Event

9.3.3.1 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".

9.3.3.2 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.

9.3.3.3 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-investigators (Dr. Flavio Frohlich and Dr. Tobias Schwippel) are experts in non-invasive brain stimulation and will provide their 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.

9.3.4 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 participant 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 participant is screened will be considered as baseline and not reported as an AE. However, if the study participant'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. 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.

Of note, common experiences associated with the application of low-amplitude electric current to the scalp such as phosphenes and tingling sensation are captured with a daily questionnaire. These events are only considered AEs if they are reported as bothersome or untoward by the participant.

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

9.3.6 Reporting of Pregnancy

Women of child-bearing potential are asked if they are pregnant or plan to become pregnant before the stimulation session. Furthermore, they are asked to use an appropriate method of birth control for the duration of trial participation. Importantly, there is no evidence that suggests tACS would interfere with pregnancy [20]. However, should a participant become pregnant during the five days of stimulation, their participation stimulation will be immediately terminated and the participant will be followed until giving birth.

9.4 Unanticipated Problems

9.4.1 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.

9.4.2 Unanticipated Problem Reporting

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

9.4.3 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 participants.

10. STATISTICAL CONSIDERATIONS

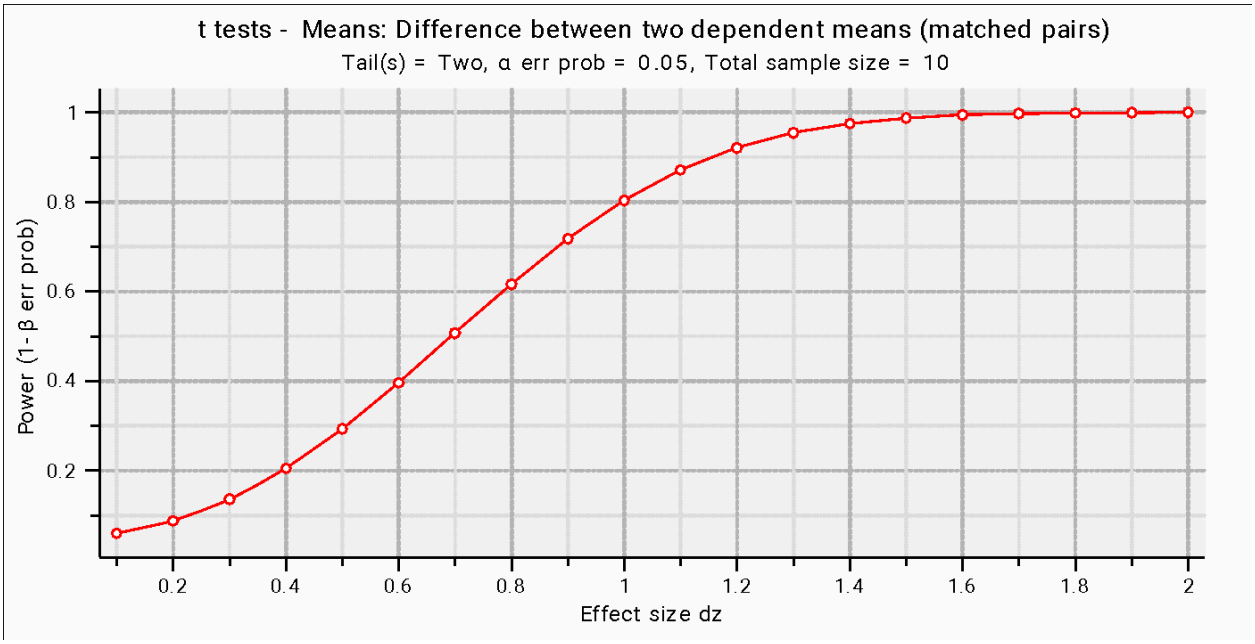
10.1 Statistical Hypotheses

Primary Efficacy hypothesis:

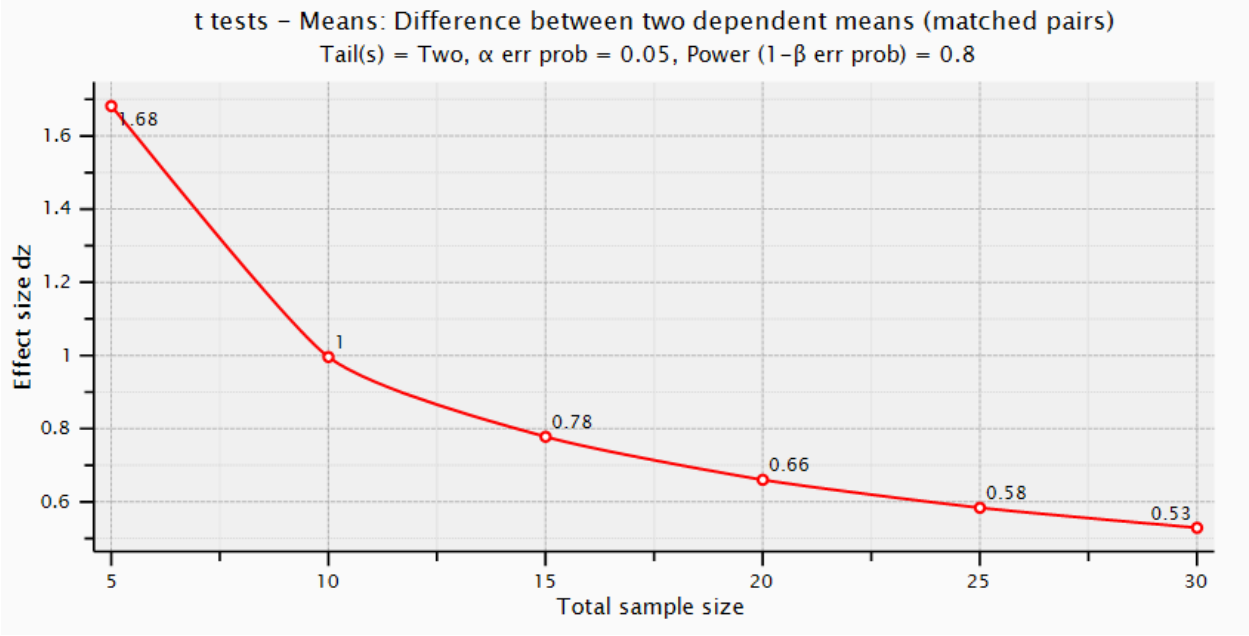
- Null: There is no difference in depression severity measured by the HDRS-17 between FU2 and Day 1.
- Alternate: There is a difference in depression severity measured by the HDRS-17 between FU2 and Day 1.

10.2 Sample Size Determination

A recently concluded pilot clinical trial compared the effects of 10 Hz tACS (therapeutic frequency) against 40 Hz tACS (control frequency). A total of 32 participants enrolled, 30 completed the 5-day intervention, and 26 completed both a 2 and a 4-week follow-up session. With approximately 9 participants in each group, a significant reduction was found in alpha oscillation power on the fifth day of stimulation ($p < 0.05$) [6], which is the presumed mechanism of action of closed-loop tACS. Therefore, with a total of 35 participants we anticipate we will find a significant difference. Further support for this sample size from this previous study derives from a Cohen's $dz = 1.58$ for change in HDRS-17 score from baseline to the 2-week follow up. For an alpha = 0.05 (two-sided) and a power of 0.95, the required sample size is N=8 based on calculation by G*power 3.1.9.7. The plot below shows how power changes as a function of effect size for a sample size of N=10. As can be seen, the current design is well-powered for the expected effect size for clinical outcomes, and adequately powered (i.e. 80%) for effects as low as $dz = 1$ for any given outcome measure.



However, the expected effects of stimulation for secondary outcome measures at FU2 are currently unclear, and self-report measures tend to have more variability and thus reduced effect sizes when compared to clinician-administered measures like the HDRS-17. Moreover, studies looking to replicate prior findings are known to require larger sample sizes compared to the original study. To that end we conducted design sensitivity analyses. We reason that a target of N = 28 for a complete dataset allows for a sound evaluation of any given effect down to dz = 0.55 which is traditionally considered in psychological research to be a medium-sized effect for two-tailed α = 0.05 at 80% power.



In the previous trial, 80% of the participants completed all study sessions. We are thus anticipating consenting around 35 people to reach the goal of 28 people for analysis. The power calculation above demonstrates what statistical conclusions will be feasible with a sample size of 28 completers.

10.3 Populations for Analyses

Every effort will be made to ensure all enrolled participants complete all study sessions as described in this protocol.

As previously stated in ***Section 7 Study Intervention Discontinuation and Participant Discontinuation/Withdrawal***, enrolled participants who do not complete the full intervention will be replaced. Therefore, with this population for analysis plan, we anticipate having data from 35 participants that are eligible for analysis.

10.4 Statistical Analyses

10.4.1 General Approach

All testing described below assumes a significance threshold of alpha = 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).

10.4.2 Analysis of the Primary Efficacy Endpoint (Depression Severity)

We will perform a paired t-test to establish the statistical significance of the change in depression symptom severity (two-sided, alpha = 0.05). Additional, strictly exploratory analyses will take into account baseline scores as covariates (e.g., degree of treatment resistance measured by the Maudsley scale).

10.4.3 Analysis of the Secondary Endpoint (RESPONSE AND REMISSION RATE, FEASIBILITY)

We will compute the response and remission rates for the sample. There is no statistical testing applicable for this outcome since there are no groups to compare.

We will compute the fraction of participants who complete stimulation by dividing the number of people who received 5-days of stimulation by the total number of participants who received any amount of stimulation.

As applicable, 95% confidence intervals will be computed to describe precision of the estimate.

10.4.4 Safety Analyses

As discussed in ***Section 8.2, Safety and Other Assessments*** safety will be assessed with the C-SSRS, an adverse effects questionnaire, and the Altman Self-Rating Mania Scale (ASRM).

The C-SSRS will be used to assess suicide risk. If a participant's responses have an elevated risk, the participant 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. The C-SSRS is administered at the screening visit and at the 14-day follow up.

The stimulation questionnaire will be administered at D1-D5 after stimulation. This questionnaire solicits ratings of 11 possible sensory experiences ("flickering light") and adverse effects ("scalp pain") associated with electrical stimulation, on a scale of 0 (absent), 1 (low), 2(medium), and 3 (high). Intensity of sensory experiences and adverse effects will be described with mean and standard deviation.

The Altman Self-Rating Mania Scale (ASRM) will be used to assess any development of mania over the course of treatment. A ASRM score of greater than 6 indicates the possible development of a manic episode. Any participant who scores greater than 6 on the ASRM will be assessed by Dr. Schiller or Dr. Rubinow. Any verified manic episode will be described in counts/percentages.

The Adverse Events Structured Interview will be administered by research personnel at D5 and whenever a participant spontaneously reports a potential AE during trial participation. This interview solicits open-ended descriptions of 13 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.*** Severity per adverse effect will be described with mean and standard deviation.

10.4.5 Baseline Descriptive Statistics

All baseline descriptive statistics will be described based on the ***General Approach described in 9.4.1***.

10.4.6 Planned Interim Analyses

There are no planned interim analyses.

10.4.7 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).

11. SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

11.1 Regulatory, Ethical, and Study Oversight Considerations

11.1.1 Informed Consent Process

11.1.1.1 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. We apply for a waiver for written consent for our pre-screening survey and our screening visit. For the pre-screening survey, interested

potential participants will indicate their consent to answering the questions as part of the survey. For the screening visit, verbal consent by the participant will be obtained and documented. Written consent for study participation will be obtained at D1. All consent forms will be IRB-approved and updated with any new information as modifications are made throughout the study.

11.1.1.2 Consent Procedures and Documentation

At the beginning of D1, 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 further explain the information. We will especially ensure that stimulation related side effects and the possibility to withdraw from the study at any given moment without prejudice are well understood.

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.

11.1.2 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 the sponsor, research staff, study participants, and the IRB and will provide the reason(s) for the termination or suspension. Study participants 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
- 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 and the sponsor.

11.1.3 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 password-protected spreadsheet. 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 NCTraCS 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 NCTraCS. 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. Access is by individual user id and is restricted to the forms and/or functions that the user needs to have.

11.1.4 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 for the duration requested by applicable law.

11.1.5 Key Roles and Study Governance

Principal Investigator	**Co-Investigator**	**Co-Investigator**	**Co-Investigator**
David Rubinow, MD	Flavio Frohlich, PhD	Crystal Schiller, PhD	Tobias Schwippel, MD
The University of North Carolina at Chapel Hill - Department of Psychiatry	The University of North Carolina at Chapel Hill - Department of Psychiatry	The University of North Carolina at Chapel Hill - Department of Psychiatry	The University of North Carolina at Chapel Hill - Department of Psychiatry
919-445-0212 david_rubinow@med.unc.edu	919-966-4584 Flavio_frohlich@med.unc.edu	919-966-4810 crystal_schiller@med.unc.edu	919-966-4584 Tobias_schwippel@med.unc.edu

11.1.6 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 as fast as possible. Based on his review, continuation of participant's participation is decided. All SAE or unanticipated AE will be reported to the local IRB and the sponsor.

11.1.7 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 participants 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.

11.1.7.1 THE CAROLINA CENTER FOR NEUROSTIMULATION MONITORING PLAN

The latest version of the approved IRB application for this clinical trial will be always followed. This responsibility falls in the hands of the trained research personnel. If at any time there is a deviation from protocol, the deviation 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 participant has completed their participation (full completion through the FU7 visit or because they withdrew prior to completion), data will be rereviewed for completeness and accuracy.

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 the responsibility of trained research personnel to report all events to the PI. Note that the study binder may assume either an electronic or paper form. Reporting of AEs and SAEs is described within ***Section 8.3***.

The PI and Co-Is (apart from Dr. Schwippel who will act as clinical rater) will have read-only access to the REDCap database. This allows the PI and Co-Is to view reports that provide information on any missing data on an individual participant basis, but does not allow them to add, change or input any data.

11.1.8 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)).

11.1.9 Data Handling and Record Keeping

11.1.9.1 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, 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 NCTraCS 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.

11.1.9.2 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 end of funding, whichever is later.

11.1.10 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.

11.1.11 Publication and Data Sharing Policy

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 a peer-reviewed journal.

11.1.12 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.

11.2 Abbreviations

AE	Adverse Event
CFR	Code of Federal Regulations

CRF	Case Report Form
DSM-5	Diagnostic and Statistical Manual, 5th Edition
eCRF	Electronic Case Report Forms
EEG	Electroencephalogram
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HDRS	Hamilton Depression Rating Scale
HIPAA	Health Insurance Portability and Accountability Act
ICH	International Conference on Harmonisation
IRB	Institutional Review Board
ISO	International Organization for Standardization
NCT	National Clinical Trial
NIH	National Institutes of Health
PI	Principal Investigator
Q-LES-Q-SF	Quality of Life Enjoyment & Satisfaction Questionnaire, Short Form
SAE	Serious Adverse Event
SHAPS	Snaith-Hamilton Pleasure Scale
SOA	Schedule of Activities
SOP	Standard Operating Procedure
UP	Unanticipated Problem

12. REFERENCES

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