Causal role of delta-beta coupling for goal-directed behavior in anhedonia

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Summary of Changes from Previous Version:

Version Control	Affected Section(s)	Summary of Revisions Made	Rationale
Version 1.2 May 13, 2021	Sections 1, 5, 8, 9	Added exploratory analysis, explained each metric in greater detail, addressed statistical feedback, fixed terminology.	Incorporated SRC feedback
Version 2 Sept 15, 2021	Section 1, 4, 5, 6, 7, 8, 9	Included new funding source: K99/R00 from NIMH awarded to JR. Removed hormone sampling. Updated screening procedure. Removed the probabilistic reward task. Split the first session into two session. Updated exclusion criteria.	New funding source is required for consent form. Hormone sampling was deemed to be not feasible. Screening procedure updated to reduce experimenter burden regarding suicide risk. Not sufficient baseline data for probabilistic reward task. Part 1 and 2 of session 1 are now separate. Further clarification on exclusion based on substance/alcohol use disorder.

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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 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.

1. PROTOCOL SUMMARY

1.1 SYNOPSIS

Title:	Causal role of delta-beta coupling for goal-directed behavior in anhedonia
Study Description:	The purpose of this clinical trial is to investigate the causal role that delta-beta coupling plays in goal-directed behavior in participants with major depressive disorder (MDD) and symptoms of anhedonia. The participants will perform a reward-based decision-making task. During the task, cross-frequency transcranial alternating current stimulation (tACS) will be delivered at delta-beta frequency, a control-frequency, or an active sham. Electroencephalography will be collected in intermittent resting-state periods. Structural and functional magnetic resonance imaging (MRI) will be collected during the resting-state and during performance of the reward-based decision-making task.
Objectives:	Primary Objective: To investigate whether delta-beta tACS will produce an increase in goal-directed behavior during reward-based decision-making. Secondary Objective: To investigate whether delta-beta tACS will result in a lasting increase in delta-beta coupling during eyes-open resting-state recorded immediately following stimulation.
Outcomes:	Primary Outcome: Behavioral metric, percentage of hard trials chosen, during the Expenditure of Effort for Reward Task (EEfRT). Secondary Outcome: Neural metric, degree of phase-amplitude coupling between prefrontal delta oscillations and left motor beta oscillations, quantified during eyes-open resting-state recorded post-stimulation intermittent with task blocks.
Study Population:	We will recruit men and non-pregnant women ages 18-65 with a diagnosis of non-psychotic MDD, free of benzodiazepines and anticonvulsant medications. Eligible participants will be stratified based on anhedonia severity such that 50% of participants must have "low" anhedonia. Participants must also have less than 3 on the Suicide Item on the Hamilton Depression Rating Scale (HAM-D) and low suicide risk determined as having no active intent as determined by the Columbia Suicide Severity Rating Scale (C-SSRS). Participants must also be eligible to receive tACS and MRI. Participants 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 use the NEUROCONN DC Stimulator Plus for investigational purposes to deliver either cross-frequency delta-beta, cross-frequency theta-gamma, or active sham transcranial alternating current stimulation. Active sham treatment will include 20 seconds of ramp in to 40 seconds of 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 participant blinding by mimicking skin sensations associated with tACS. Theta-gamma and delta-beta tACS will also have a 20 second ramp in and ramp out for each task block. With 8 total task blocks each lasting 5 minutes, participants receive a total of 40 minutes of stimulation. Stimulation waveform is a low-frequency sine wave with a superimposed high-frequency sine-wave at the peak of each cycle. The zero-to-peak amplitude of stimulation is 2mA.
Study Duration:	2 years
Participant Duration:	Participation for each participant will be 2 weeks. Completion includes three sessions: a clinical assessments session, a stimulation session, and an MRI session. The clinical assessments session will take approximately 2 hours. The stimulation session will take approximately 3 hours. The visit to the MRI takes approximately 1.5 hour. We estimate that total participation to be approximately 6.5 hours.

1.2 SCHEMA

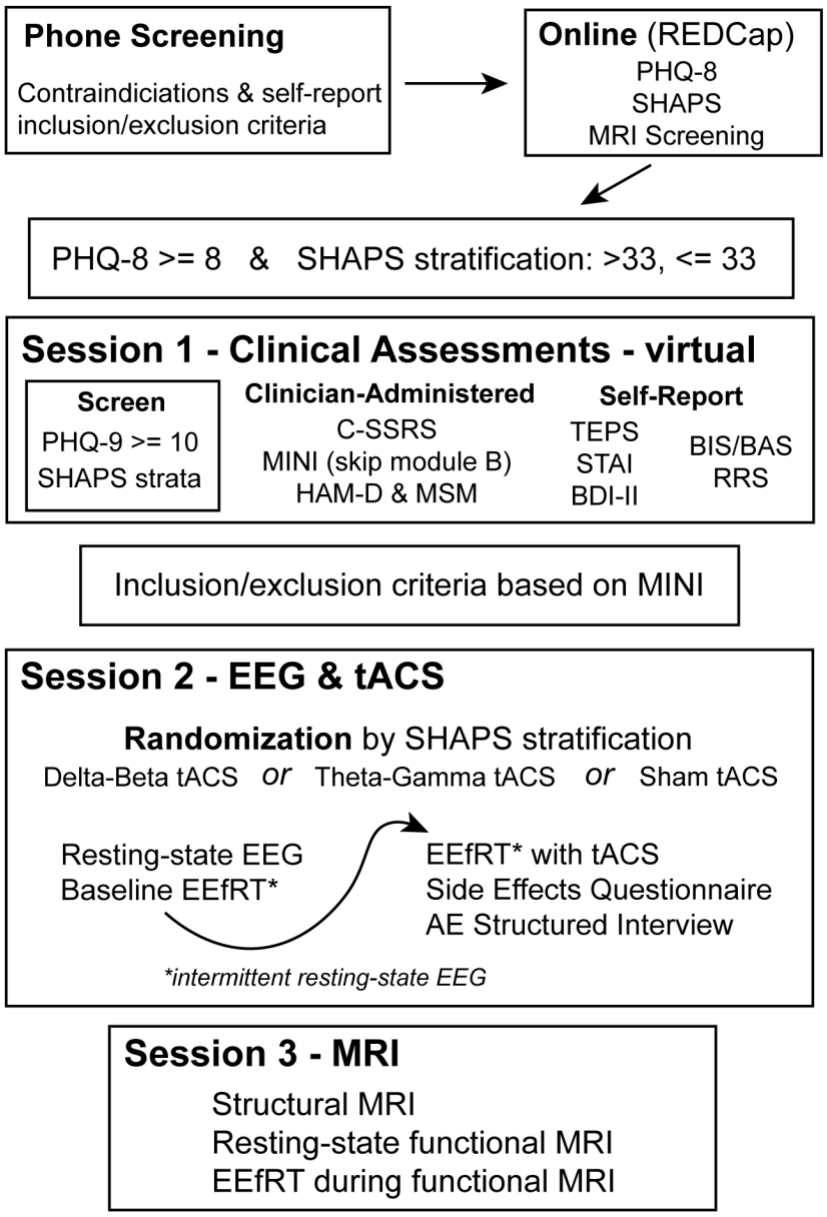

Figure 1. Experimental design depicted as a flow-chart.

1.3 SCHEDULE OF ACTIVITIES (SOA)

Procedures	Phone Screening	Online Assessments	Session 1 Clinical Assessments	Session 2 - Stimulation	Session 3 - MRI
Informed consent	X (verbal)	X (digital)	X (written)		
Determine Eligibility	X	X	X		
PHQ-8 (without suicide q.)		X			
PHQ-9			X		
SHAPS		X	X	X	
Demographics			X		
Medical history			X		
MINI (diagnostic)			X		
C-SSRS			X		
Maudsley Staging Method			X		
HAM-D			X		
RRS			X		
BIS/BAS			X		
SHAPS			X		
C-SSRS			X		
TEPS			X		
Beck Depression Inventory - II			X		
BAS/BIS			X		
STAI			X		
Urine Pregnancy Test				X	
Randomization				X	
Resting-state EEG				X	
EEfRT				X	
EEfRT with tACS				X	
Intermittent resting-state EEG				X	
Stimulation Questionnaire				X	
AE Structured Interview				X	
AE Review and Evaluation				*	
Structural MRI					X
Resting-state MRI					X
Functional MRI with EEfRT					X

* when applicable

2. INTRODUCTION

2.1 STUDY RATIONALE

Anhedonia, the inability to seek-out and experience pleasure, remains difficult to treat, with many pharmacological interventions occasionally increasing, or introducing, anhedonia (Treadway and Zald 2011, Husain and Roiser 2018, Lambert, Da Silva et al. 2018). Many proven treatments in psychiatry are less effective in patients with elevated symptoms of anhedonia (Nierenberg, Keefe et al. 1999, Shelton and Tomarken 2001, Landén, Högberg et al. 2005, Hatzigiakoumis, Martinotti et al. 2011, McMakin, Olino et al. 2012). In affective disorders such as major depressive disorder (MDD), anhedonia is positively correlated with suicidal behavior (Bonanni, Gualtieri et al. 2019). Therefore, effective treatment for anhedonia is a critical factor in the ongoing mental health crisis. Symptoms of anhedonia fall within the "Positive" domain of the Research Domain Criteria (RDoC) framework (Nusslock and Alloy 2017) and comprise two primary constructs (Gard, Gard et al. 2006, Kring and Barch 2014). The "liking," or consummatory, component is the experience of reward as pleasurable. Collectively, reward evaluation is known to critically rely on the orbitofrontal cortex of the medial prefrontal cortex (mPFC) to process the value of the reward (O'doherty-Schoppe and Cai 2011) and the nucleus accumbens of the ventral striatum (vSTR) to evaluate the effortful cost (Sugam, Day et al. 2012). Correlational studies find reduced activation and decreased functional connectivity in mPFC-vSTR during reward-based decision-making tasks in patients with anhedonia (Epstein, Pan et al. 2006, Wacker, Dillon et al. 2009, Greenberg, Chase et al. 2015). The second component is the "wanting," or anticipatory, component that reflects the benefit computation –*goal-directed behavior*. The dorsal striatum (dSTR) processes the anticipation of receiving a future reward and orients behavior towards reward-seeking (Pizzagalli, Holmes et al. 2009, Zhang, Lin et al. 2016). The dSTR (caudate and putamen) is guided by the dorsolateral prefrontal cortex (dlPFC; middle frontal gyrus) and together they play a critical role in planning and initiating goal-directed behavior (Badre and Nee 2018). Lesion to the dlPFC results in deficits in planning and initiating goal-directed behavior (Szczepanski and Knight 2014) and lesion to dSTR results in apathy and similar deficits as lesions to dlPFC (Mendez, Adams et al. 1989). Thus, the neuropsychological etiology of anhedonia presents two candidate neural circuits tied to two relevant cognitive constructs: mPFC-vSTR for reward-evaluation and dlPFC-dSTR for goal-directed behavior (Kring and Barch 2014, Rizvi, Pizzagalli et al. 2016, Höflich, Michenthaler et al. 2019). Pathology of both circuits is *correlated* with anhedonia (Walsh, Eisenlohr-Moul et al. 2019), and causal investigation may differentiate the relative importance of each circuit.

2.2 BACKGROUND

In the past few decades, non-invasive brain stimulation emerged as a promising intervention for treatment-resistant depression that is safe with minimal side-effect (Perera, George et al. 2016). The most widely available stimulation protocols to treat depression use transcranial magnetic stimulation (TMS) to increase neural activity in the left dlPFC (Perera, George et al. 2016) or mPFC (Downar, Geraci et al. 2014, Siddiqi, Taylor et al. 2020), and recent work from our lab found promising treatment effects using transcranial alternating current stimulation (tACS) to dlPFC (Alexander, Alagapan et al. 2019, Riddle, Rubinow et al. 2020). With multiple spatial targets emerging for the treatment of depression, researchers are beginning to understand that heterogeneity in depression symptoms alters which spatial targets are most effective at treating depression (Siddiqi, Taylor et al. 2020). Inaccurate spatial targeting can render treatment ineffective (Fox, Buckner et al. 2012). Therefore, improved understanding of the neural circuits that underly specific symptoms of depression is of critical need. One such symptom, anhedonia, is more responsive to stimulation targeting dlPFC than stimulation targeting mPFC (Downar, Geraci et al. 2014,

Drysdale, Grosenick et al. 2017, Duprat, Wu et al. 2018, Pettorruso, Spagnolo et al. 2018, Siddiqi, Taylor et al. 2020). Stimulation to left dlPFC in depressed patients with anhedonia only alleviated depression when symptoms of anhedonia decreased (Levkovitz, Sheer et al. 2011). In contrast, stimulation to mPFC only alleviated depression in patients without symptoms of anhedonia (Downar, Geraci et al. 2014). Despite recent evidence that dlPFC-dSTR is the optimal target for treating anhedonia in depression, the overall response rate is still lower than depressed patients without anhedonia (Krepel, Rush et al. 2020). One explanation is that stimulation efficacy is also critically dependent on the temporal structure of neural activity (Ali, Sellers et al. 2013). Thus, investigation of the electrophysiology of dlPFC-dSTR during goal-directed behavior in patients with anhedonia is critical to further advance the efficacy of treatment interventions.

Neural oscillations are a critical mechanism for interregional communication (Fries 2015), with low frequency oscillations facilitating long-distance communication and high frequency enhancing local connectivity (Buzsáki, Anastassiou et al. 2012). Cognitive control tasks evoke low frequency neural oscillations in prefrontal cortex that couple to high frequency oscillations in posterior cortex (Canolty and Knight 2010, Voytek, Canolty et al. 2010, Voytek, Kayser et al. 2015, Helfrich, Huang et al. 2017). This *cross-frequency coupling* is proposed to be a mechanism for prefrontal cortex to exert top-down control (Canolty and Knight 2010). One candidate cross-frequency coupling signal for goal-directed behavior is the coupling of delta oscillations (2-4Hz) in prefrontal cortex to beta oscillations (15-30Hz) in motor cortex (Wyart, de Gardelle et al. 2012, Riddle, McFerren et al. 2021). In the proposed experiment, we investigate the causal role of delta-beta coupling and the dlPFC-dSTR neural circuit in goal-directed behavior. We are convinced that our experiment is of high significance since a mechanistic understanding of the neural circuits that underlie goal-directed behavior will ultimately allow the rational design of novel brain stimulation techniques to more effectively treat depressed patients with anhedonia.

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 and including the informed consent document are kept in locked filing cabinets in locked rooms separate from any source documents containing participant dummy identifiers. The document that links study ID numbers to personal identifying information is encrypted and protected using a password-protected document on a secure server provided by UNC School of Medicine. 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. 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. 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 over a decade without any report of serious side effects (Antal, Alekseichuk et al. 2017). 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 (Frohlich and McCormick 2010). However, tACS does have some mild side effects, such as transient mild tingling, burning, or itching under the electrode sites. In our previous trial, participants 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" (Alexander, Alagapan et al. 2019). 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 present during these sessions will also check in with the participant periodically during the stimulation to see whether they are comfortable. If participant 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 tACS will in any way increase this likelihood. In a previous clinical trial that administered tACS for five consecutive days in a treatment paradigm, 4 participants in the sham/placebo stimulation group experienced an increase in suicidal thoughts and only one of those 4 participants reported suicidal intent (Alexander, Alagapan et al. 2019). No participants in this previous trial who received tACS reported an increase in suicidal ideation from baseline. Regardless, participants with high suicide risk 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 Suicide Item included in the HAM-D (Snaith, Hamilton et al. 1995)to assess suicide risk, as well as the Columbia Suicide Severity Rating Scale (C-SSRS) (Posner, Brown et al. 2011)to determine intent for suicide during the enrollment eligibility interview. Inclusion criteria state that the participant must be low suicide risk and that potential participants with an above "low risk" eligible for the study. In the event that suicide risk is determined in the first session, the participant will be withdrawn from the study and will be provided with a referral to UNC Department of Psychiatry, and their mental health care or family medical doctor will be contacted.

Risk of Psychological Discomfort: There is a psychological element in that some participants may become claustrophobic upon entering the small space of the MRI bore. To reduce psychological distress, participants are informed that they can withdraw consent and stop participation at any time. Participants are monitored throughout the MRI scans and can terminate the scan at any time by squeezing a ball held in the hand, and will be quickly removed from the MRI bore.

Risk of Injury from MRI: Magnetic resonance imaging (MRI) is a non-invasive imaging modality with an outstanding safety profile in the United States. The risk of physical injury from the presence of metallic objects within the body is almost entirely addressed through the use of screening forms, by requiring participants to change into medical gowns, and by using metal detectors at the site of the MRI. Through using a core facility at UNC-CH, the Biomedical Research Imaging Center (BRIC), we ensure that risks are minimized as BRIC technicians independently screen and prepare participants for imaging.

2.3.2 KNOWN POTENTIAL BENEFITS

This study has not been designed to benefit the individual participants. However, the knowledge gained from this study will contribute to understanding about the psychological and biological basis of major depressive disorder. Furthermore, the results from this study might be used to develop future interventions using non-invasive brain stimulation.

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.

2.3.4 REFERRALS FOR MEDICAL FOLLOW-UP

Epileptic Activity Follow-up: There is a theoretical likelihood that stimulation of neuronal circuits can lead to epileptic discharge. To minimize this occurrence, we screen and exclude participants with personal and family history of neurological conditions from the study. We further emphasize that there has never been a single report of a seizure that resulted from transcranial alternating current stimulation or transcranial direct current stimulation. If abnormalities or a seizure is witnessed during the course of the study, a referral will be made to Clio Rubinos at UNC Department of Neurology for follow-up. In the theoretical event that a seizure is witnessed that involves the loss of consciousness, the participants will be told not to operate a motor vehicle until cleared by the DMV.

Neurological Abnormality Follow-up: When imaging the brain there is a chance that the MRI scan will reveal a neurological irregularity that might be of medical importance to the participant. These incidental findings are uncommon and rarely lead to early identification of neurological issues. However, as is standard practice, we allow participants the option to choose to be alerted to any incidental findings. In the consent form, participant can opt out of being notified about incidental findings. If a researcher notices a neurological abnormality, then they will reach out to our collaborators in the department of neurology to investigate the MRI scan further. If the neurologist considers the abnormality to be of medical importance, then the participant will be contacted for future steps. It should be noted that the research MRI scans used (T1-weighted) are not diagnostic scans and are not sensitive to detecting common neurological problems such as brain cancer. There is no expectation that the participant population studied in this research program will be of greater likelihood for incidental finding.

3. OBJECTIVE AND OUTCOME MEASURES

OBJECTIVES	OUTCOMES	JUSTIFICATION FOR OUTCOMES
Primary		
To investigate whether delta-beta tACS will produce an increase in goal-directed behavior during reward-based decision-making.	Behavioral metric, percentage of hard trials chosen, during the Expenditure of Effort for Reward Task (EEfRT) at Session 1.	Stimulation is delivered during task performance and the amount of time on task with stimulation was sufficient to observe meaningful changes in goal-directed behavior across participants in our previous study.
Secondary		

OBJECTIVES	OUTCOMES	JUSTIFICATION FOR OUTCOMES
To investigate whether delta-beta tACS will result in a lasting increase in delta-beta coupling during eyes-open resting-state recorded immediately following stimulation	Neural metric, degree of phase-amplitude coupling between prefrontal delta oscillations and left motor beta oscillations, quantified during eyes-open resting-state recorded post-stimulation intermittent with task blocks.	Our previous study delivering stimulation in the same manner with intermittent resting-state EEG found evidence of increased phase-amplitude coupling from stimulation.
Exploratory		
To investigate the degree to which motivation symptoms predict individual differences in the impact of delta-beta tACS on performance.	Two-dimension factor analysis is run on clinical assessments to derive a motivation symptoms and mood symptoms dimension. These scores are correlated with the impact of tACS on behavior.	Our previous study found two symptom dimensions within participants with major depressive disorder that captured individual differences in reward-based decision-making.
To investigate whether rewarding learning might be impaired in addition to goal-directed behavior in participants with MDD and anhedonia.	Optimal decision-making for rewarding stimulus is predicted to be impaired as a function of anhedonia and may be improved with delta-beta tACS.	Previous literature suggests that reward-learning is impaired in patients with depression and symptoms of anhedonia. This additional task allows for the investigation of the specificity of tACS to influence a specific reward-based cognitive process.
To investigate whether functional connectivity between dorsal striatum and left lateral prefrontal cortex predicts individual differences in the impact of delta-beta tACS on performance.	Functional connectivity analysis of resting-state functional magnetic resonance imaging is correlated with the impact of tACS on behavior.	Previous research has suggested that frontal-striatal connectivity is related to reward-based decision-making. Participants with anhedonia may show reduced connectivity within this neural circuit that is predictive of the impact of tACS on reward-based decision-making.

4. STUDY DESIGN

4.1 OVERALL DESIGN

This study is a pilot, three-session, parallel-arm study with transcranial alternating current stimulation (tACS), electroencephalography (EEG), and magnetic resonance imaging (MRI) to understand the causal role of delta-beta coupling in goal-directed behavior in participants with major depressive disorder (MDD) and symptoms of anhedonia. Participants that request to be in the experiment will provide verbal, documented consent to undergo a phone screening to assess that the participant meets exclusion/inclusion criteria. Then, participants will complete demographic and self-report clinical assessments via REDCap: Patient Health Questionnaire-8 (PHQ-8) with the suicide question removed (Kroenke, Spitzer et al. 2001) and the Snaith Hamilton Pleasure Scale (SHAPS) (Snaith, Hamilton et al. 1995). Participants that have a PHQ-8 greater than or equal to 8 will be invited to participate in the

study. Also, participants will be stratified based on their SHAPS score (50% > 33 on the SHAPS and 50% <= 33). Stratification is conducted to ensure a representative spread in anhedonia scores since we are interested in the relationship between anhedonia and goal-directed behavior. Thus, some participants will not be invited to participate if we have already completed sessions for 24 participants within their median split. The value of 8 for the PHQ-8 is used to help ensure that participants will meet criteria for MDD; thus, improving the feasibility of the study. The value of 33 for the SHAPS was the median SHAPS score from our previous experiment in participants with MDD, and is consistent with findings from other groups. While the PHQ-8 and SHAPS are used to determine eligibility (SHAPS is exclusionary only if a stratum is full), additional self-report clinical assessments are administered for exploratory analyses.

The first session will be a virtual clinical assessments session. Participants will provide verbal consent including a brief verbal assessment of understanding of study procedures. Next, participants will complete two assessments on REDCap while being monitored in a virtual session: the PHQ-9 and must score at least a 10 to proceed with the full session, and the SHAPS is used to check if their stratum is full for randomization purposes. After passing this additional screening, self-report clinical assessments will be completed on REDCap while being monitored: Behavioral Activation System and Behavioral Inhibition System (BIS/BAS) (Carver and White 1994), Temporal Experience of Pleasure Scale (TEPS) (Gard, Gard et al. 2006), the State-Trait Anxiety Inventory (STAI) (Spielberger 2010), Beck's Depression Inventory version 2 (BDI-II) (Beck and Beamesderfer 1974), and Ruminative Responses Scale (RRS) (Nolen-Hoeksema, Larson et al. 1999). In addition, participants will undergo clinician-report assessments that will be entered onto REDCap: the Mini International Psychiatric Interview for the DSM-V (MINI) (Sheehan, Lecrubier et al. 1998), Hamilton Depression Rating Scale (HAM-D) (Hamilton 1960), Columbia-Suicide Severity Rating Scale (C-SSRS) (Posner, Brown et al. 2011), and the Maudsley Staging Method (MSM) (Donaldson, Poon et al. 2009). Eligibility for the experiment is then determined by a MINI diagnosis of MDD, exclusion based on a present moderate to severe substance use disorder or alcohol use disorder, a history of severe substance use disorder or alcohol use disorder, or a diagnosis of psychosis or psychotic features. In addition, the participant must have low suicide risk as determined by the C-SSRS (see Inclusion/Exclusion criteria above). In the event that a participant has active plans to commit suicide, an adverse event is recorded, an acute psychiatric assessment is conducted by our medical monitor, Dr. Crystal Schiller, who is a clinical psychologist at UNC-CH, and participation is terminated. In the case that the person does not see anyone for their depression, Dr. Schiller will assist the participant in seeking medical care. Dr. Rubinow (Principal Investigator, mood disorders expert) will be available as well, in any instances where Dr. Schiller is unavailable for acute assessment.

In the second session, participants provide written consent to participate in the study. Then, participants are randomized into one of three arms of the study: delta-beta tACS, control tACS in theta-gamma, or active sham. Randomization is stratified by SHAPS level such that there are an equal number of participants that are high anhedonia (SHAPS > 33) and low anhedonia (SHAPS <= 33). We expect that of the 48 participants in the final dataset, there will be 8 from each of the 6 groups (3 stimulation conditions by 2 anhedonia levels). Two 4.5 by 4.5 centimeters stimulation pads are applied to the scalp over the left hemisphere anterior and ventral to F3 and posterior and ventral to C3, and a 5 by 7 centimeters stimulation pad is applied centered over FCz in an anterior to posterior direction. Prior to any task performance, participants under eyes-closed and eyes-open resting-state while fixating on a central point on the monitor. Participants complete titration of the difficult of the EEfRT. Then, participants perform 4 blocks of the EEfRT during the collection of EEG. After each block of the EEfRT, there is a 2 minute eyes-open resting-state scan. Next, participants receive tACS during performance of the EEfRT. Delta-beta tACS, theta-gamma tACS, or sham-tACS is received during task performance. Stimulation is turned off between blocks, and eyes-open resting-state EEG is acquired. The total amount of stimulation is approximately 40 minutes for 8 blocks that

each last approximately 5 minutes. After tACS, participants complete a stimulation side effects questionnaire and a blinding questionnaire. A blinding questionnaire is completed by the experimenter. The blinding questionnaire is a single question that asks whether the participant receive stimulation or placebo, and then asks the participant to provide an explanation for their response. Based on the results of the stimulation side effects questionnaire, a structured adverse events interview is conducted to acquire more information regarding any side effects that were selected to be "very high" by the participant.

For the third session, participants are instructed to arrive at the Biomedical Research Imaging Center (BRIC) in Marsico Hall on UNC-CH campus. Participants are instructed to arrive at the facility 30 minutes early to ensure that they are ready to go into the MRI for the 60-minute allotted timeslot. During the 60 minutes of scanning, a 5-minute structural MR is acquired, 10-minutes of functional MR scans during the eyes-open resting-state are acquired, and the remaining time is used to complete as many blocks of the EEfRT as possible. Completion of the MRI session is not required for the dataset to be considered a complete dataset. These additional data acquisitions enable exploratory analyses that will be used to design future studies.

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 participants) will be blind to the participant's arm assignment until all data has been collected. This will reduce implicit and explicit bias in the data collection process. In this study, participants will be randomized in to one of three arms: active sham (i.e., placebo) stimulation, delta-beta tACS, or theta-gamma tACS. This is a follow-up to two of our previous studies. One study delivered this same form of stimulation in participants during a cognitive control task, which is different from the reward-based decision-making task described here. This study used a similar task design in which intermittent resting-state EEG found that delta-beta tACS and theta-gamma tACS increased the targeted neural activity and modulated behavior associated with the targeted neural activity. In this experiment, participants and researchers were successfully blinded to which stimulation condition they received. Furthermore, this previous study was a crossover design and, thus, a parallel arm design is even more likely to be double-blind because participants only receive one form of stimulation. A second study recorded EEG during the EEfRT in patients with MDD. This study found that participants with MDD and higher symptoms of anhedonia demonstrated reduced goal-directed behavior and reduced delta-beta coupling. This preliminary data provides strong evidence that stimulation delivered in a delta-beta pattern should be beneficial to behavioral performance. Furthermore, this study serves as a target engagement study and future studies may use these results to develop interventions to treat symptoms of anhedonia in MDD.

4.3 JUSTIFICATION FOR DOSE

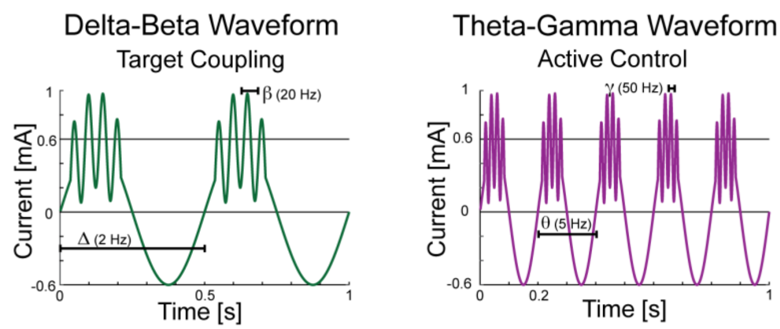

Figure 2. Electrical waveforms for delta-beta tACS (left) and theta-gamma tACS (right). Peak stimulation amplitude is 1 mA for target electrodes.

Transcranial alternating current stimulation (tACS) is an extremely safe non-invasive stimulation paradigm without a single serious adverse event directly related to stimulation. The same tACS method has been used during performance of a task with intermittent resting-state EEG in our previous approved UNC IRB protocol 18-0003 (NCT03800030) that was overseen by Dr. Riddle. A similar experimental design using a single session with reward-based decision-making tasks and tACS in participants with major depressive disorder (MDD) was approved in our previous UNC IRB protocol 16-1911 (NCT03449979) that was overseen by Dr. Riddle. Transcranial Alternating Current Stimulation (tACS) applies a weak electric current to the scalp and all previous studies (approximately 15 to date) performed in the Carolina Centers ifgonri fNiecuarnots trimsukl adteisoing UNC IRB. Thus, no direct FDA oversight is required and no investigational device application filing is required. Further conversations with the regulatory core of the UNC CTSA and the director of the Office of Human Research Ethics that administers the UNC IRB over the years have consistently confirmed this classification.

tACS will be delivered using a cross-frequency stimulation waveform delta-beta (3-20Hz), a control frequency of theta-gamma (5-50Hz), or sham in which stimulation is delivered in either delta-beta or theta-gamma for 10 seconds and then returns to baseline (Figure 1). These waveforms were designed based on our previous experiment that observed these brain activity patterns in a reward-based decision-making task in participants with MDD. The stimulation amplitude delivered is standard for tACS studies (Ahn, Mellin et al. 2018, Alexander, Alagapan et al. 2019) –stimulation peaks at 1 mA during the peak of the high-frequency component for target electrodes (see Y-axis of Figure 2). The high frequency component consists of 3.5 cycles of a sine-wave of the high frequency that is centered at 90 degrees of each cycle of the low-frequency component. Stimulation peaks at 2 mA zero-to-peak for the return electrode, but the electrode size is 35 cm^2 for the return electrode and 20.25 cm^2 for each of the target electrodes. Thus, the current density is comparable between the two: 0.057 mA/cm^2 for the return electrode and 0.049 mA/cm^2 for the target electrodes.

4.4 END OF STUDY DEFINITION

The end of this study is defined as when the last participant completes the study (i.e., the 48th participant to complete their final session).

5. STUDY POPULATION

5.1 INCLUSION CRITERIA

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

- Between the ages of 18 and 65
- Able to provide informed consent
- Have normal to corrected vision
- Willing to comply with all study procedures and be available for the duration of the study
- Speak and understand English
- Low suicide risk as determined by both the Mini International Neuropsychiatric Interview (MINI) for the DSM-5 (assessment of suicidality) and the Columbia Suicide Severity Rating Scale (C-SSRS; "low risk" no for item five regarding intent to act), and by the Hamilton Depression Rating Scale (HAM-D; less than 3 for the suicidality item).
- Negative pregnancy test for female participants
- PHQ-8 greater than or equal to 8 prior to the first session
- PHQ-9 greater than or equal to 10 at the start of the first session
- A diagnosis of major depressive disorder on the MINI

5.2 EXCLUSION CRITERIA

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

- ADHD (currently under treatment)
- Neurological disorders and conditions, including, but not limited to:
    - History of epilepsy
    - Seizures (except childhood febrile seizures)
    - Dementia
    - History of stroke
    - Parkinson's disease
    - Multiple sclerosis
    - Cerebral aneurysm
    - Brain tumors
- Medical or neurological illness or treatment for a medical disorder that could interfere with study participation (e.g., unstable cardiac disease, HIV/AIDS, malignancy, liver or renal impairment)
- Prior brain surgery
- Any brain devices/implants, including cochlear implants and aneurysm clips
- History of current traumatic brain injury
- (For females) Pregnant or breast feeding
- 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
- DSM-V diagnosis of present moderate or severe substance use disorder or alcohol use disorder, and past severe substance use disorder or alcohol use disorder, or psychotic disorder within the last 12 months
- Not taking medications for ADHD or benzodiazepines as these medications often produce specific EEG activity that may disrupt our interpretation of the findings
- If major depressive disorder is experienced in episode, the participant must currently be within a depressive episode.
- Contraindications for MRI: ferrous metal inside the body, jewelry must be removable, pacemaker or cochlear implant.

5.3 LIFESTYLE CONSIDERATIONS

None.

5.4 SCREEN FAILURES

In the design of this study, initial phone screening procedures should identify the majority of participants who could potentially become screen failures if consented to participate in the clinical trial. However, the phone screening process does not necessarily account for all exclusion criteria. After the phone screening, each participant is provided with a link to complete two online assessments via REDCap: the Patient Health Questionnaire (PHQ-8, without the suicide question) and the Snaith-Hamilton Pleasure Scale (SHAPS). Participants must have a PHQ-8 greater than or equal to 8, because this will help to ensure that participants will be diagnosed with MDD during session 1. Also, the stimulation is stratified based on SHAPS (>33 or <=33). Thus, if 24 participants have completed either of the stratum, then participants within that stratum will not be eligible. In the case that a participant meets these criteria, there is still a chance that the first interview reveals that they do not meet study criteria. The study personnel completing the interviewing process will clearly explain why the participant does not meet criteria. However, in the case that a participant does not qualify based on suicide risk, procedures will be followed to ensure participant safety.

At the first session and after obtaining participant informed consent, the HAM-D will be administered, which contains a question related to suicidal thoughts/actions. If someone answers greater than 2 on the HAM-D (i.e., either "suicidal ideas or gesture" or "attempts at suicide") and indicates intent based on the C-SSRS, their participation in the study will be immediately stopped and Dr. Schiller (Co-I, responsible for participant safety) will be contacted for acute assessment. In the case that the person does not see anyone for their depression, Dr. Schiller will assist the participant in seeking medical care. Dr. Rubinow (Co-Investigator, mood disorders expert) will be available as well, in any instances where Dr. Schiller is unavailable for acute assessment.

Assessment may include facilitating contact of the participant with their psychiatrist/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 will assist the participant in establishing care. If at any point in the assessment, the participant is deemed to be an imminent risk of harm to self or others, study personnel will Department for further care. In any instances where Dr. Schiller is unavailable, Dr. Rubinow will be available to assist the participant. Dr. Rubinow will be the final arbitrator in case anything (e.g., eligibility, safety) is unclear during this process. Dr. Schiller and the researcher will defer to Dr. Rubinow in those instances.

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 d
and Raleigh areas. We estimate that approximately 75 participants will be enrolled from the Chapel

Hill/Carrboro area and 25 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. Interested individuals can than call or email the secure line/address to set up a phone prescreening. 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.

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, 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. We may also be launching a Facebook ad to identify potential patients. This ad will also use a pre-screening survey via REDCap to help identify participants. We will also be using the UNC Mass email listserv to send out an email that has the studypages.com link. 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.

Retention is primarily achieved by condensing the bulk of the experiment into a single stimulation session. The payment is $30 for completion of virtual clinical assessments session. Then, completion of the stimulation during task provides an addition $50. Finally, participants are invited back for a third session that is an MRI session which provides $40. Thus, completion of all study requirements gives $120. However, completion of the in-person stimulation session is sufficient to address all primary and secondary outcomes. The participant will receive payment at the end of each session on a payment card that can be loaded. The research staff will also give each participant a reminder call or email for upcoming sessions. Each research staff member will be available for the participants to contact via email or phone.

6. STUDY INTERVENTION

6.1 STUDY INTERVENTION(S) ADMINISTRATION

6.1.1 STUDY INTERVENTION DESCRIPTION

Participants will be stimulated with the commercial, CE-certified Neuroconn Plus stimulator. The use of this device in this study has previously received a NSR designation on initial review by the full UNC IRB. The NeuroConn device description is as follows:

The DC-STIMULATOR is a CE-certified medical device for conducting non-invasive transcranial direct-current stimulation (tDCS) in humans. DC stimulation is used in clinical practice and in the research of stroke, epilepsy, migraine, tinnitus, depression, multiple sclerosis, dementia and chronic headache. The DC-STIMULATOR is a micro-processor-controlled constant current source. It meets the highest safety standards thanks to (hardware- and software-based) multistage monitoring of the current path. By continuously monitoring electrode impedance it can detect insufficient contact with the skin and automatically terminate stimulation, maximizing patient safety.

The device's alphanumeric display and the 4 touch keys allow various stimulation modes to be selected and stimulation parameters such as current strength, duration, fade-in and fade-out to be set.

DC-STIMULATOR features:

- 1 channel (anodal and cathodal stimulation possible)
- Adjustable current up to 5,000 µA *
- Adjustable application time up to 30 minutes *
- 2 standard modes - single (continuous stimulation) and - pulse (cyclical stimulation activation/deactivation) with fade in and fade out
- Customer-specific programs possible (optional)
- "Study mode" for blind processing of genuine and 'pseudo' stimulation (optional)
- External trigger input (optional)

6.1.2 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 4.5x4.5cm electrodes placed anterior and ventral to F3 and posterior and ventral to C3, and one 5x7cm electrode placed over FCz. Electrodes will be carbon rubber, with Ten20 conductive paste applied. During stimulation, the participant will be performing the EEfRT. The stimulator will be triggered by remote control from the experiment script and will administer stimulation for five-minutes during each of the 8 task blocks for a total of 40 minutes of stimulation. After each block of stimulation, an eyes-open resting state EEG will be performed. All stimulation involves 20 seconds of ramp-in time and 20 seconds of ramp-out time.

The stimulation waveforms used in this study for active sham stimulation (placebo) and for delta-beta and theta-gamma tACS are delivered at 1 mA zero-to-peak amplitude at the target electrodes and 2 mA zero to-peak amplitude at the return electrode. For active sham stimulation, the stimulation is left on for only 40 seconds before the stimulation ramps back out. 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 participant's assignment.

Codes will be randomized to one of the three experimental arms. Researchers will enter the participant specific code into the MATLAB script that control the stimulator. Researchers will monitor participants during stimulation. 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
Mengsen Zhang, a Frohlich Lab member, will oversee the randomization of 48 6-digit codes, which will be used by the experimenters. These codes are directly linked to which treatment participants receive

(sham/placebo or 10 Hz tACS at 1 mA) and will be entered into the MATLAB script. 24 codes are generated for each of the anhedonia stratum. In addition, 12 codes will be generated (6 extra codes for each stratum) in the event that a participant is randomized but discontinues the study. Thus, data will be collected until we reach out target of 48 participants, but there will be no bias towards completion for the different arms (in the unlikely event that one of the arms has more dropout than the others).

The assignment of each participant cannot be determined by looking at the codes (e.g., codes are not sequential, code assignment is not based on "odd" vs "even" number). Thus, the study is double-blind randomized and concealed by virtue of using random numbers generated by a computer. Mengsen Zhang has no other responsibility in the study other than providing these randomized codes. If Mengsen Zhang leaves the Frohlich Lab, another equivalent researcher who does not work with human participants will perform this task.

Blinding
This study is designed to be double-blind. This means that the participant and the researchers are unaware of each participant'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 active sham condition includes some stimulation, mimicking the skin sensations associated with tACS. In our previously concluded trial, participants in the delta-beta tACS, theta-gamma tACS, and active sham groups responded similarly to the blinding questionnaire, indicating that our active sham stimulation successfully blinded the participants.

6.4 STUDY INTERVENTION COMPLIANCE

Full compliance with the intervention is defined as completing the entire first session, the MRI session, and providing at-home saliva samples. As the intervention is applied and monitored by research personnel, compliance can be directly observed.

6.5 CONCOMITANT THERAPY

Eligible participants will be permitted to be receiving concomitant therapy, such as therapy, antidepressants, or other medications. Given that the current trial investigates target engagement acutely from stimulation, there is no investigation in changes of symptoms. Thus, exploratory analyses will consider individual differences that can be explained by differences in concomitant interventions. The only medications not permitted during this trial are anticonvulsants and recent benzodiazepines. The use of antidepressants or other therapies is unlikely to affect our outcome, as our primary and secondary outcomes are with respect to a single session.

Concomitant therapies will be logged at the first session. 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.

6.5.1 RESCUE MEDICINE

N/A

7. STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTION

Discontinuation of stimulation during session 1 means that study participation is halted and no other sessions are completed. If a clinically significant finding is identified after enrollment, the investigator or qualified designee will determine if any change in participant management is needed. Any new clinically relevant finding will be reported as an adverse event (AE).

The study intervention (i.e., 40 minutes of stimulation spaced by intermittent resting-states) will be discontinued for the following reasons:

- 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).

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

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

An investigator may discontinue or withdraw a participant from the study for the following reasons:

- 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).

The reason for participant discontinuation or withdrawal from the study will be recorded with the participant files. Participants who sign the informed consent form and are not randomized will be replaced. Participants who sign the informed consent form, are randomized, and receive the full or part of the study intervention (40-minutes of stimulation on session 2), and subsequently withdraw from the study, are withdrawn from the study, or discontinue the study will not be replaced. However, participants who sign the informed consent form, and are not randomized 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 1 scheduled visit and is 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 MRI session, research personnel will be flexible in timing, including offering this session later in the day as well as some weekends. Furthermore, the at-home saliva sample can be returned at any time.

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 session will be labeled as missing data and our pre-determined analysis plan takes into consideration missing data. Of note, neither the primary nor secondary outcomes are dependent on the MRI session or at-home saliva samples.

8. STUDY ASSESSMENTS AND PROCEDURES

8.1 EFFICACY ASSESSMENTS

Inclusion and exclusion criteria will be determined at the initial session, including concomitant therapies, medical history, and diagnosis, to ensure that participants are diagnosed with MDD, with low suicide risk, and free of benzodiazepines and anticonvulsant medications.

8.1.1 ELECTROPHYSIOLOGY

1. Resting-state EEG (RSEEG) recordings will be completed several times during the second session. Eyes-open and eyes-closed RSEEG will be collected before any task are performed or stimulation is delivered. Then, after every task block without and with stimulation, two-minutes of eyes-open RSEEG are collected. This measure is used to determine the immediate after-effects of tACS on brain activity, specifically on phase-amplitude coupling between delta oscillations in prefrontal electrodes and beta oscillations in left motor electrodes and between theta oscillations in prefrontal electrodes and gamma oscillations in bilateral parietal-occipital electrodes.
2. Phase-amplitude coupling between delta-beta and theta-gamma oscillations will be acquired during task performance of the EEfRT as measured in our previous study (Riddle, Alexander et al., in review) and similar to our previous study with delta-beta tACS and theta-gamma tACS (Riddle, McFerren et al. 2021).

8.1.2 TASK PERFORMANCE

1. Goal-directed behavior: In the Expenditure of Effort for Reward Task (EEfRT), goal-directed behavior is quantified as the percentage of trials in which the participant chose the HARD task instead of the EASY task. In our previous experiment, goal-directed behavior negatively and significantly correlated with anhedonia symptom severity. Goal-directed behavior ranges from 0 to 100% and task difficulty is titrated to push goal-directed behavior down to <85%. In our previous use of this task, minimum goal-directed behavior was 23%. Thus, we expect performance to range from approximately 23-85%.
2. Reward-evaluation: In the EEfRT, reward-evaluation is quantified as the slope of the linear fit of percentage HARD trials for each incentive level ($2.50 to $6 in $0.50 increments), dependent variable, to the incentive level, independent variable. In our previous experiment, reward-evaluation positively and significantly correlated with trait anxiety severity in participants with depression. In our previous experiment, the average reward evaluation was 15% per $1.

8.1.3 CLINICAL EVALUATIONS

1. MINI INTERNATIONAL PSYCHIATRIC INTERVIEW FOR THE DSM-V (MINI) is a structured interview that is an abbreviation of the Structured Clinical Interview for the DSM (Sheehan, Lecrubier et al. 1998). The MINI is ideal for the diagnosis of MDD and to be used as screening technique for exclusion diagnoses. These data are used to determine eligibility after the first session.
2. HAMILTON DEPRESSION RATING SCALE (HAM-D) is a 17-item assessment that determines depression severity (Hamilton 1960). This assessment is used to complement the BDI-II. Items within the HAM-D have been shown to capture different dimensions of MDD, such as anhedonic and anxiosomatic symptoms. This scale is also used to determine suicide risk. Items are summed to a total score ranging 0 to 54.
3. COLUMBIA-SUICIDE SEVERITY RATING SCALE (C-SSRS) is used to screen for suicide risk (Posner, Brown et al. 2011). In the event that a participant reports plans or intention to commit suicide, then an acute psychiatric assessment is conducted by Dr. Crystal Schiller.
4. MAUDSLEY STAGING METHOD (MSM) is a method for measuring treatment resistance in participants with depression on a scale of 3 to 15 (mild = 3-6; moderate = 7-10; severe=11-15)(Donaldson, Poon et al. 2009).

8.1.4 SELF-REPORT ASSESSMENTS

1. The Beck Depression Inventory (BDI) (Spielberger et al. 1983) will be administered at session 1. The BDI is a 21-item, self-report rating inventory that measures characteristic attitudes and symptoms of depression. Scores range from 0 to 63.
2. The Behavioral Inhibition and Behavioral Activation Self Report Scales (BIS/BAS) (Carver and White 1994) will be completed during the first session. These scales are used to monitor the perceived sensitivity to reward and punishment. BIS/BAS is broken into four sub-scores: BIS (7 to 28), BAS drive (4-16), BAS fun-seeking (4-16), and BAS reward (7-20).
3. The State-Trait Anxiety Inventory (STAI) (Spielberger et al. 1983) will be completed during the first session. These 40 items are used to quantify frequencies of feelings of anxiety. STAI has two subscores, state and trait anxiety, range 20 to 80.
4. The Snaith-Hamilton Pleasure Scale (SHAPS) (Snaith, Hamilton et al. 1995) will be completed online before the first session to stratify randomization and again at the first session. This scale is used to assess anhedonia, range 14 to 56.
5. Patient Health Questionnaire-9 (PHQ-9) is a 9-item assessment that mirrors the criteria for major depressive disorder (Kroenke, Spitzer et al. 2001). This assessment is used as a screening tool that is completed prior to the first in-person session to increase the feasibility of the study by only running sessions with participants that will most likely be diagnosed with MDD, an inclusion criterion. Range 0 to 27.
6. Temporal Experience of Pleasure Scale (TEPS) is an 18-item assessment that quantifies symptoms of anhedonia along two sub-dimensions: consummatory (8 to 48) and anticipatory (10 to 60) (Gard, Gard et al. 2006). This assessment may provide additional insight into subtypes of anhedonia symptoms and is acquired during the first session.
7. Ruminative Responses Scale (RRS) is a 22-item assessment that quantifies the degree to which participants engage in depressive rumination, range 31 to 155 (Nolen-Hoeksema, Larson et al. 1999). This assessment is completed at the first session.

8.2 SAFETY AND OTHER ASSESSMENTS

1. The Columbia Suicide Severity Rating Scale (C-SSRS) (Posner, Brown et al. 2011) will be administered by trained research personnel at the first session to thoroughly assess suicide risk. If a participant reports experiencing either suicidal ideation or suicidal behavior, 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 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 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 an acute assessment is required, then participation will be halted.
2. A structured interview probing for the experience of adverse events based on the responses from the stimulation side effects questionnaire will be completed after stimulation on the second session. Dr. Frohlich, 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.
3. A stimulation side effects 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 participant 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 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 da therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily eq

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 concomitant events). Although an AE may rate o can be flagged as requiring more info rmation and later be upgraded "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., clinical condition, other concomitant treatments). It's clini
- **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. Frohlich), with input from the co-investigators (Dr. Schiller and Dr. Rubinow) when necessary, will determine whether an adverse event (AE) is expected or unexpected in this population. The principal investigator (Dr. Flavio Frohlich) is an expert in non-invasive brain stimulation and will provide his expert opinion in regard to 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 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. 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, 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. At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

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

There have been some scientific studies using transcranial electrical stimulation in pregnancy (see (Kurzeck, Kirsch et al. 2018) and (Konstantinou, Vigod et al. 2020) for review). None of these studies reported side-effects specific to pregnancy or fetal development. Furthermore, given the history of an extremely safe profile for non-invasive electrical stimulation with zero serious adverse events after decades of use, we assume there to be no risk to pregnant women. Nonetheless, pregnant women will be excluded from this study because they are a protected group. Female participants will be asked if there is a possibility that they are pregnant at both sessions. If the participant says yes or is unsure, then we will verify pregnancy status via a urine pregnancy test. Only upon a verbal confirmation that pregnancy is not possible or a negative finding from a pregnancy test will we proceed with the experiment.

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 (i.e., 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 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.

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 participant.

9. STATISTICAL CONSIDERATIONS

Statistical analyses will be performed by Dr. Justin Riddle.

9.1 STATISTICAL HYPOTHESES

- Primary Outcome hypothesis:
  - Null: There is no difference in goal-directed behavior between baseline EEfRT and EEfRT during stimulation as a function of stimulation type (delta-beta tACS, theta-gamma tACS, or active-sham).
  - Alternate: There is a difference in goal-directed behavior between baseline EEfRT and EEfRT during stimulation as a function of stimulation type (delta-beta tACS, theta-gamma tACS, or active-sham).

- Secondary Outcome hypothesis:
  - Null: There is no difference in delta-beta phase-amplitude coupling strength between prefrontal electrodes and left motor electrodes for post-stimulation resting-state periods as a function of stimulation type (delta-beta tACS, theta-gamma tACS, or active-sham).

- Alternate: There is a difference in delta-beta phase-amplitude coupling strength between prefrontal electrodes and left motor electrodes for post-stimulation resting-state periods as a function of stimulation type (delta-beta tACS, theta-gamma tACS, or active-sham).

- Exploratory Outcome hypothesis 1:
  - Null: The impact of delta-beta tACS on goal-directed behavior is not correlated with motivation symptom severity.
  - Alternative: The impact of delta-beta tACS on goal-directed behavior is correlated with motivation symptom severity.

- Exploratory Outcome hypothesis 2:
  - Null: Functional connectivity during the resting-state between dorsal striatum and lateral prefrontal cortex is not predictive of the impact of delta-beta tACS on goal-directed behavior.
  - Alternative: Functional connectivity during the resting-state between dorsal striatum and lateral prefrontal cortex is predictive of the impact of delta-beta tACS on goal-directed behavior.

9.2 SAMPLE SIZE DETERMINATION

The sample size is 48 participants in the final dataset to be used in analysis. However, to ensure that 48 participants complete the experiment, we conservatively estimate to enroll 100 participants as a ceiling for the sake of IRB approval. The motivation for the sample size of 48 participants in the analysis is explained below. The sample size is determined based on previous experiments from our group: the effect size of previous findings and the reliability of our estimates. Our estimators are precise due to sufficient number of trials per condition with each participant and that the tasks are titrated to the individual.

Primary outcome: Cross-frequency transcranial alternating current stimulation (tACS) to lateral frontal cortex at delta-beta frequency will increase goal-directed behavior relative to placebo stimulation.

Previous Data: The planning and refinement of the proposed study was informed by our previous experiments that serve as preliminary data for the proposal. In our previous experiment, we delivered cross-frequency tACS to lateral frontal cortex at delta-beta frequency to modulate performance on a cognitive control task relative to placebo stimulation (Riddle, McFerren et al. 2021). As in the current proposal, a specific component of the task was identified to drive an increase in delta-beta coupling. This was the component that was altered by delta-beta tACS. In the previous experiment, we found a significant interaction between stimulation (delta-beta tACS or theta-gamma tACS, minus placebo) and our cognitive control dimension of interest with a partial-eta square of 0.288 with N = 23. For the specific comparison of interest, we found increased reaction time relative to placebo with an effect size of 0.480 from pair-wise t-test with N = 23.

Sample Size Determination and Power Calculation: Our previous effect size based on behavioral modulation from delta-beta tACS used a crossover design. The experiment proposed here will use a parallel arm design; however, we will calculate the effect of stimulation as a difference from the baseline

performance levels that are derived from our titration of task difficulty. Therefore, the primary analysis will use a mixed-measures ANOVA with one within-participants factor (before and during stimulation) and one between-participants factor (delta-beta, placebo). With an effect size of 0.288 partial-eta square, we estimate that we need at least 21 participants to reach 80% statistical power with a correlation of 0.7. Using a conservative estimate of 0.5 correlation between estimates, we still reach 93% statistical power with 48 participants.

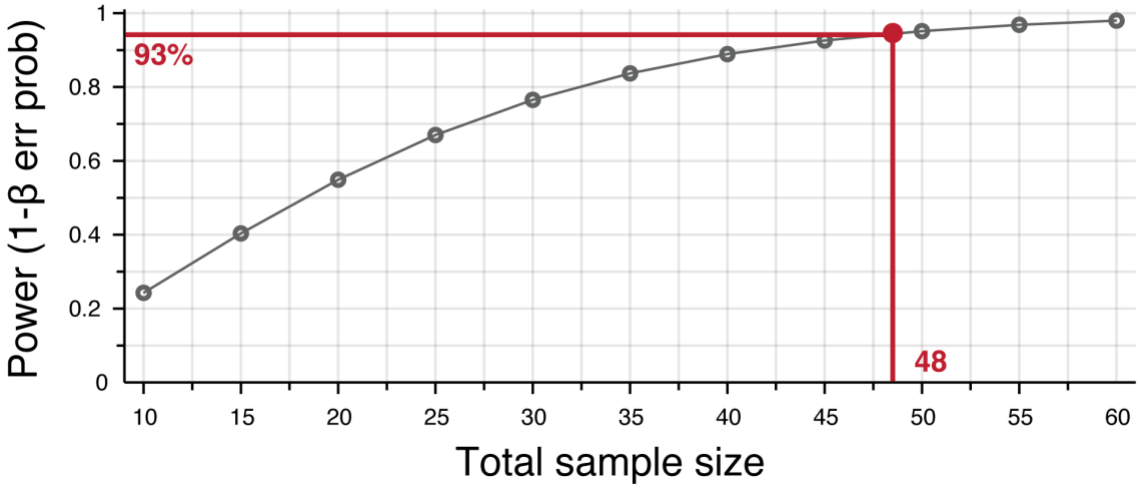

Figure 3. Power calculation for the impact of delta-beta tACS on performance tested with ANOVA: between-participants factor of stimulation type and within-participants factors before versus after stimulation. Interaction effect was modeled as a within-between ANOVA using the G*Power software: 3 groups, 2 measurements, partial-eta square 0.288, correlation between estimates conservatively at 0.5. Proposed number of participants, 48, is depicted in red.

Data will be collected until we reach 48 participants. Given the similarity in stimulation methodology between the current experiment and our previous experiment (Riddle, McFerren et al. 2021), we anticipate adequate levels of power for hypothesis testing with 48 total participants.

Secondary outcome: Cross-frequency transcranial alternating current stimulation (tACS) to lateral frontal cortex at delta-beta frequency will increase delta-beta coupling relative to placebo stimulation.

Previous Data: The planning and refinement of the proposed study was informed by our previous experiment that serves as preliminary data for the proposal. In our previous experiment, we delivered cross-frequency tACS to lateral frontal cortex at delta-beta frequency to modulate performance on a cognitive control task relative to placebo stimulation (Riddle, McFerren et al. 2021). As in the current proposal, resting-state EEG sessions were included between each task block such that phase amplitude coupling could be estimated with data that was not corrupted by the stimulation artifact from tACS. In

the previous experiment, we found that delta-beta tACS increased delta-beta coupling with an effect size of 0.382 and a sample size of 23 participants.

Sample Size Determination and Power Calculation: Our previous effect size based on the modulation of delta-beta coupling from delta-beta tACS used a crossover design. The experiment proposed here will use a parallel arm design; however, we will calculate the effect of stimulation as a difference from the baseline coupling levels that are derived from resting-state EEG prior to stimulation. Therefore, the primary analysis will use a mixed-measures ANOVA with one within-participants factor (before and during stimulation) and one between-participants factor (delta-beta or placebo). With an effect size of 0.382, we estimate that we need at least 21 participants to reach 80% statistical power. With 48 participants, then we expect power to exceed 95% with a conservative estimate of 0.5 correlation between estimates.

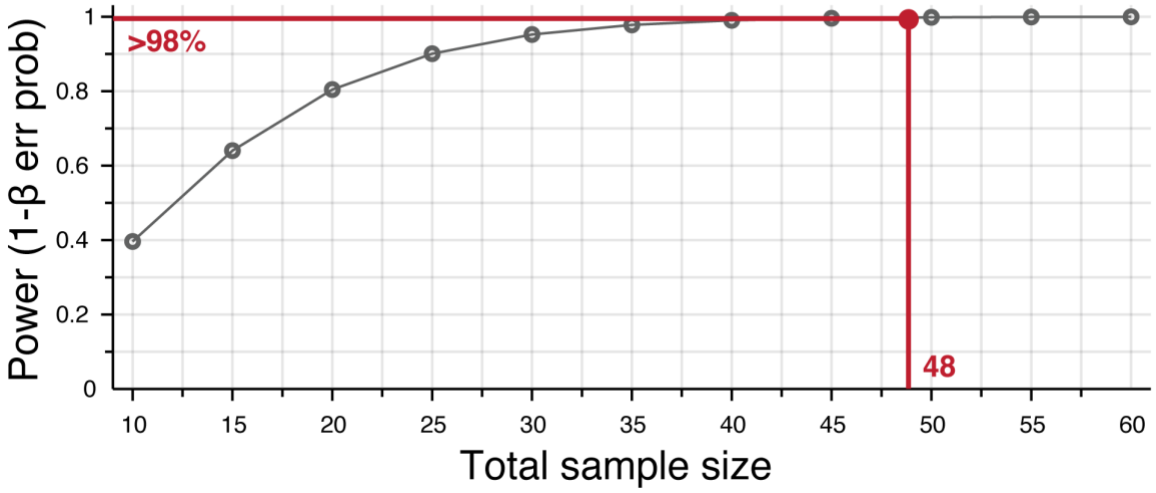

Figure 4. Power calculation for the impact of delta-beta tACS on delta-beta coupling. Interaction effect was modeled as a two-way ANOVA with factors: within-participant before or after stimulation and between-participant factor of stimulation type (delta-beta tACS, theta-gamma tACS, or placebo) using the G*Power software. Number of groups = 3, number of measurements = 2, effect size = 0.382, correlation between estimates conservatively at 0.5. Proposed total number of participants is 48 depicted in red.

Data will be collected until we reach 48 participants. Given the similarity in stimulation methodology between the current experiment and our previous experiment (Riddle, McFerren et al. 2021), we anticipate adequate levels of power for hypothesis testing with 48 total participants.

9.3 POPULATIONS FOR ANALYSES

Every effort will be made to ensure all enrolled and randomized participants 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 participants will be randomized to receive 40 minutes of stimulation. If a participant completes the intervention (i.e., completes the first session), they will be included in all analyses moving forward.

As previously stated in ***Section 7 Study Intervention Discontinuation and Participant Discontinuation/Withdrawal***, enrolled participants who do not complete session 1 will be replaced. Therefore, with this population for analysis plan, we anticipate having data from 48 participants 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. Analyses will be deemed to be statistically significant if the p-value is less than this threshold. An analysis that does not exceed this threshold will be considered inconclusive. Continuous data will be described using means, standard deviations, and confidence intervals, while categorical data will be described using counts/percentages.

There may be additional covariates included in the analysis. Data will be assessed for normality and, if deemed necessary, corrective procedures will be applied (e.g., log normalization). Based on our previous dataset, we expect that the variables used here will be approximately Gaussian. However, if the distribution of a variable displays a skewed tail and a test for normality fails, then it is justified to use a corrective procedure. This correction will be applied upon consideration of the variables themselves, and not based on the result of the intended analysis.

Data will be collected until 48 participants complete the first session and all data is usable. Given that there is only a single session, we expect to have complete data and all analyses will include the full participant pool. However, if there are technical errors that are not caught in time, then the affected analyses may have less participants. In this scenario, the data for a participant is then completely removed from the analysis. We emphasize that lost data is typically of greater concern for longitudinal and multi-session studies. If a subset of participants does not complete the second session in this experiment, then only the analysis of exploratory outcome 2 will be affected.

9.4.2 ANALYSIS OF THE PRIMARY OUTCOME (BEHAVIOR)

Goal-directed behavior will be calculated as the average decision to perform the HARD task. The average for the four blocks prior to stimulation and the four blocks during stimulation will be calculated. A two-way analysis of covariance (ANCOVA) will be performed using within-participant factor of time (before or during stimulation), between-participant factor stimulation type (delta-beta tACS, theta-gamma tACS, or active-sham), and anhedonia symptom severity as a linear covariate (SHAPS at session 1 as a continuous variable). We hypothesize to find an interaction between time and stimulation type in the ANCOVA. Post-hoc differences will be investigated using Tuke
hypothesize that the predicted interaction will be driven by an increase in goal-directed behavior for delta-

beta tACS. Sensitivity analyses will be conducted to evaluate the specificity of these effects: a similar analysis with reward-evaluation as the dependent variable and we do not expect that delta-beta tACS will have any effect on reward-evaluation.

Furthermore, we hypothesize that the inclusion of the SHAPS covariate is critical to our findings in that participants with the greatest symptoms of anhedonia will demonstrate the greatest increase to goal-directed behavior. This effect will be investigated by a correlation between the baseline symptoms of anhedonia (SHAPS) and the change in goal-directed behavior for participants that received delta-beta tACS. The specificity of this effect will be investigated as a difference in correlation between the relationship of anhedonia symptoms and goal-directed behavior for the group that received sham tACS.

9.4.3 ANALYSIS OF THE SECONDARY OUTCOME (ELECTROPHYSIOLOGY)

Delta-beta phase amplitude coupling will be calculated between the phase of delta oscillations (2-3 Hz) in prefrontal electrodes (FCz and surrounding electrodes) and the amplitude of beta oscillations (15- 25 Hz) in left motor electrodes (C3 and surrounding electrodes). The instantaneous phase and amplitude of these oscillations will be calculated by averaging the signal in these two regions of interest, band-filtering the signal to the specified range, and then performing the Hilbert transform on the signal. Phase-amplitude coupling (PAC) is then calculated by creating a hybrid signal using the amplitude of beta oscillations in left motor electrodes and the phase of delta oscillations in prefrontal electrodes:

B, M is magnitude of beta oscillations θ is an points

The PAC value is normalized by creating a null distribution by randomly shifting the beta timeseries by at least 10% of the number of time points. Then, PAC is calculated between the delta-phase timeseries and each of these randomly shifted beta-amplitude timeseries. Finally, PAC_Z is calculated as the z-transformed true PAC value relative to the null distribution.

The average for the eight resting-state period after each task block prior to stimulation and the eight resting-state periods after each task block during stimulation will be calculated. A two-way analysis of covariance (ANCOVA) will be performed using within-participant factor of time (before or during stimulation), between-participant factor stimulation type (delta-beta tACS, theta-gamma tACS, or active-sham), and anhedonia symptom severity as a linear covariate (SHAPS at session 1 as a continuous variable). We hypothesize to find an interaction between time and stimulation type. Post-hoc differences will be investigated using Tukey's method to add

predicted interaction will be driven by an increase in delta-beta coupling for delta-beta tACS. Sensitivity analyses will be conducted to evaluate the specificity of these effects: a similar analysis with theta-gamma coupling as the dependent variable and we do not expect that delta-beta tACS will have any effect on theta-gamma coupling. In addition, we will perform an analysis on the topographic specificity of the increase in delta-beta coupling across the scalp. We hypothesize that the increase in delta-beta coupling will be specific to the left motor electrodes.

9.4.4 ANALYSIS OF THE EXPLORATORY OUTCOMES

First exploratory outcome: symptom dimensions

A two-dimension factor analysis will be run on all sub-scores from clinical assessments to derive a mood and motivation composite symptom score. This analysis was used in our previous study and found that goal-directed behavior negatively correlated with motivation symptoms. Thus, we hypothesize that the severity of motivation symptoms will correlate with the degree to which delta-beta tACS will increase goal-directed behavior. The analysis will be run as a Pearson correlation within the group that received delta-beta tACS. To control for the specificity of this effect, this correlation will be compared to sham tACS, mood symptoms, and reward-evaluation.

Second exploratory outcome: functional connectivity in frontal-striatal circuity

Using the resting-state functional magnetic resonance imaging data, we will calculate the strength of functional connectivity between the dorsal striatum and the lateral prefrontal cortex. We hypothesize that participants with major depressive disorder and anhedonia will show decreased strength in connectivity, and that individual differences in this functional connectivity strength will be predictive of the change in goal-directed behavior with delta-beta tACS. The analysis will be run as a Pearson correlation within the group that received delta-beta tACS. To control for the specificity of this effect, this correlation will be compared to sham tACS and reward-evaluation.

9.4.5 SAFETY ANALYSES

The stimulation questionnaire will be administered for the first session after stimulation. This questionnaire solicits ratings of 14 possible adverse effects associated with electrical stimulation, on a scale of 1 (low) to 4 (very high). Paired t-tests with random effect "participant" will be calculated per adverse effect to determine if there are any differences in adverse effect severity between groups (delta-beta tACS versus active-sham, theta-gamma tACS versus active-sham). Severity per adverse effect will be described with mean and standard deviation.

The Adverse Events Structured Interview will be administered by research personnel following the stimulation session. This interview solicits open-ended descriptions of each of the symptoms that were rated to be "very A linear model with random effect "participant" will be calculated per adverse effect to determine if there are any differences in adverse effect severity between groups (delta-beta tACS, theta-gamma tACS, active-sham tACS as categorical independent variables) and adverse effect severity as the continuous dependent variable. Severity per adverse effect will be described with mean and standard deviation.

9.4.6 BASELINE DESCRIPTIVE STATISTICS

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

9.4.7 PLANNED INTERIM ANALYSES

If there is an unexpected event that is related to tACS, then a blinded interim descriptive analyses on the safety measures (blinded adverse effects and responses to the stimulation questionnaire) will be performed. If there is a reason to review unblinded information, a researcher not involved in the data collection for the study, Mengsen Zhang or another researcher in the group, will be access the list of

participant identification numbers kept by Mengsen Zhang. Participant identification numbers will be in a table according to the two arms of the study; however, the treatment of each arm will not be disclosed. This information will allow the researcher to compare the two groups.

There are no other planned interim analyses.

9.4.8 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 p
and continues throughout the individual's study p
benefits of tACS will be provided to the participants and their families. Consent forms describing, in detail, the study intervention, device, procedures, and risks are given to the participant and written documentation of informed consent is required prior to the administration of any treatment. Electronic consent will be documented via REDCap before the initial interview assessments. 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

During a phone call, 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. Participants must complete the eConsent documentation via REDCap prior to any additional procedures taking place. If needed, the participants will have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. Participants will be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice.

At the beginning of the first visit to the lab, participants will sign a physical copy of the consent document witnessed by research personnel. A copy of the signed informed consent document will then be given to the participant for their records. 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 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.

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 testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence.

All research activities will be conducted in as private a setting as possible.

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 or an encrypted server maintained by the Carolina Center for Neurostimulation.

10.1.5 KEY ROLES AND STUDY GOVERNANCE

Principal Investigator	**Co-Investigator**	**Co-Investigator**
Flavio Frohlich, PhD	David Rubinow, MD	Crystal Schiller, PhD
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
919-966-4584 Flavio_Frohlich@med.unc.edu	919-445-0212 David_Rubinow@med.unc.edu	919-966-4810 Crystal_Schiller@med.unc.edu

10.1.6 SAFETY OVERSIGHT

Safety oversight will be under the direction of the principal investigator and co-investigators composed of three clinical researchers. The PI and/or Co-I will review AEs in real time and make decisions as of participation of the clinical trial. The PI will review AEs as appropriate, every three months at a minimum, with the research team. The PI may request additional review by Co-I on a case-by-case basis.

10.1.7 CLINICAL MONITORING

The purpose of the monitoring plan is to present the approach of the Carolina Center for Neurostimulaton 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.

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 from protocol log will be filled out. All team members will be trained on how and when to use this log. Deviations will be sent to IRB every 4-6 weeks (if necessary).

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 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 Medical School Wing C, Room 233. 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 participant 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 and biological specimen collection, documentation and completion. Following written Standard Operating Procedures (SOPs), research personnel will verify that the clinical trial is conducted and data are generated, 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. The REDCap data management system will be programmed and overseen by Justin Riddle.

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 C schedule for General Records Retention and Disposition Schedule, 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. Protocol deviations will be sent to the IRB per their guidelines. The site PI/study staff will be responsible for knowing and adhering to their IRB requirements.

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/Adverse Experience
ANOVA	Analysis of Variance
BDI-II	Beck's Depression Inventory
BIS/BAS	Behavioral Inhibition System / Behavioral Approach System
BRIC	Biomedical Research Imaging Center
CFR	Code of Federal Regulations
Co-I	Co-Investigator
CRF	Case Report Form
C-SSRS	Columbia Suicide Severity Rating Scale
DHHS	Department of Health and Human Services
dlPFC-dSTR	Dorsolateral prefrontal cortex to dorsal striatum
DMV	Department of Motor Vehicles
DSM-5	Diagnostic and Statistical Manual of Mental Disorders, 5th Edition
EEfRT	Expenditure of Effort for Reward Task
EEG	Electroencephalogram
EMG	Electromyography
FDA	Food and Drug Administration
fMRI	Functional magnetic resonance imaging
GCP	Good Clinical Practice
HAM-D	Hamilton Depression Rating Scale
HD-EEG	High-density electroencephalography
Hz	Hertz
ICF	Informed Consent Form
IDE	Investigational Device Exemption
LAR	Legally Authorized Representative
mA	Milliamperes
MDD	Major depressive disorder
MINI	Mini International Neuropsychiatric Interview
mPFC-vSTR	Medial prefrontal cortex to ventral striatum
MSM	Maudsley Staging Method
MRI	Magnetic Resonance Imaging
NIH	National Institutes of Health
NIMH	National Institute of Mental Health
NSR	Non-significant risk
OHRE	Office of Human Research Ethics
OHRP	Office for Human Research Protections
PAC	Phase-amplitude coupling
PHI	Protected Health Information
PHQ-9	Patient Health Questionnaire with 9 items
PI	Principal Investigator
RDoC	Research domain criteria
RRS	Ruminative responses scale
SAE	Serious Adverse Event/Serious Adverse Experience
SHAPS	Snaith-Hamilton Pleasure Scale

SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure
STAI	State-Trait Anxiety Inventory
tACS	Transcranial Alternating Current Stimulation
TEPS	Temporal Experience of Pleasure Scale
UE	Unexpected Event
UNC	University of North Carolina
UNC-CH	University of North Carolina at Chapel Hill
US	United States

10.4 PROTOCOL AMENDMENT HISTORY

MAINTAINED AT THE TOP OF THIS DOCUMENT

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