

Pilot study using endogenous aperiodic brain activity to personalize transcranial alternating current stimulation as a treatment for antenatal depression: PandA-tACS

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**Pilot study using endogenous aperiodic brain activity
to personalize transcranial alternating current
stimulation as a treatment for antenatal depression:
Panda-tACS**

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Summary of Changes from Previous Version:

Affected Section(s)	Summary of Revisions Made	Rationale
From V7		
Inclusion Criteria	Changed inclusion criteria to mild-severe depression (HDRS>8)	Excluding individuals with mild depression is unnecessary because the severity of depressive symptoms will not influence the study's primary outcomes (feasibility)
Prescreen	Removed DIAMOND screening questions	We have found that participants often did not complete these questions, and the same questions are asked at the clinician screening visit.
Recruitment materials	- Added additional recruitment blurb - Amended flyer to add new UNC logo and language that stimulation is non-invasive	This blurb will enable further sharing of studies on websites
From V6		
Inclusion Criteria	Removed the requirement for a preidentified DSM-5 diagnosis of unipolar, nonpsychotic MDD.	- Reduces prescreen ineligibility by basing inclusion on current clinical presentation rather than documented history. - Screening interviews will confirm if participants meet current diagnostic criteria for depression.
Prescreen	Remove EPDS from prescreen	This extra questionnaire was originally added to get more information about current depression symptoms, however it has been noted to increase participant burden such that participants are not

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		completing the prescreen. The study team screens for current depression symptoms (HDRS-17) during screen, thus EPDS is not needed
Recruitment	• Clarified methods of contact after completion of prescreening survey	• Ensure patient-facing compliance
From V5:		
Outcomes	• Feasibility outcome in Phase II antenatal depression: changed to “<i>The number of participants who complete all study visits within the intervention period in the antenatal depression group [Timeframe: Baseline to D5]</i>” (i.e., removed FUP1 and FUP2) • Separated FUP1-FUP2 (postnatal) as a separate exploratory outcome (rather than grouping with antenatal outcomes)	• This was an error corrected to align with the stated justification for endpoint, i.e., feasibility of the intervention (not the feasibility of follow-up). • FUP1-FUP2 are postnatal follow-up timepoints. As the study is primarily focused on the antenatal period, this follow up was separated into an independent timepoint.
From V4:		
Procedure	• EPDS asked as part of prescreen • Remove all DIAMOND self report screening questions except exclusionary modules	• Determines level of depression before clinician screen • Lowers prescreen burden for participant as data is not relevant to the antenatal depression arm (previously included for HCs)
Devices	• Added neuroConn LOOP-IT device as back-up device option	• The neuroConn LOOP-IT is the newest version of the DC Stimulator MC. It has been added to the protocol as a backup device.

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From V3:		
Procedures	Screenings will be recorded on HIPAA-compliant zoom	Ensure clinician inter-rater reliability
From V2 (participant recruitment not yet commenced):		
Assessments	- Removed likert mood scale - Added three new likert scales: anxiety, tiredness, sleep quality	Removed mood scale as this information is already captured through mania check, HDRS, EPDS Added new scales to further capture heterogeneity in study sample, e.g. due to potential modulating effects of sleep, anxiety, tiredness.
	Mania and C-SSRS questions wording 'since last visit' for D3, D5, FUP	Mania check and C-SSRS already asked in D1. The same question was asked again in error on D3, D5, FUP. Reworded to capture these since the last visit.
	Removed question about self harm in D1	Self-harm 'in the last two years' queried in screening (D -1 to -30). This question was included again in error on D1.
	- Additional DIAMOND modules added (previously only a concise version of DIAMOND) - Added DIAMOND self-report screener to prescreen additionally for antenatal depression group	Previously, only inclusionary/exclusionary DIAMOND modules included. Including all DIAMOND modules will ensure that all possible comorbid diagnoses are captured. DIAMOND self-report screener was originally only added for HC. Including DIAMOND self-report screener in prescreen for antenatal depression participants allows study team to review inclusionary/exclusionary answers before inviting to screening.
	BP measurement additionally added after stimulation for antenatal depression group and both before and after for HC group	BP will be measured after stimulation for antenatal depression, in addition to prior to stimulation. BP will be measured both before and after stimulation for the HC group as a

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		reference point for the antenatal depression group.
	New REDcap electronic case report forms attached	Added to capture changes above
Intervention	Amended stimulation delivery from 8 x 5 min blocks (with 1 min EEG in between) to 4 x 10 minute blocks (with 2 min EEG in between)	This change allows longer periods of EEG to be recorded (2 min rather than 1 min), providing a more stable EEG recording
From V1:		
Endpoints/statistics	- Minor updates to endpoint description - Added follow-up at 4-6 weeks post-birth (FUP2), mirroring FUP1	Minor endpoint updates made to align with clinicaltrials.gov published entry Follow-up added to investigate association between antenatal and postnatal depression.
Intervention	- Participants will watch Reefscapes video during HD-EEG - Electrode montage altered from 2x circular ring electrodes to 3x square electrodes - Change length of sessions from 3.5 hrs D1, D5 and 1.5 hr D2-4, to 2-2.25 hours each day	Updates made after preliminary waveform testing and more in depth electric field modelling Updates to session timing made after mock sessions
Schema	- Add option to complete baseline EEG session to D1 - Updated session timings: - Healthy controls (Phase I): Fourteen hours across seven visits over four weeks. - Antenatal depression (Phase II): Sixteen hours across eight visits over 15-33 weeks (depending on gestational age at treatment)	This is practically feasible and will decrease participant burden. Session timings updated after extra follow-up added
Assessments	Add 'date of last menstrual cycle' to demographics form for HCs	Updates made to increase pregnancy safety monitoring

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	• Add exports of all redcap forms • Add DIAMOND self report screening questions to prescreen for healthy controls	RedCap for data collection newly completed Add self report screening to prescreen to increase efficiency of screening
Study documents	• Added Certificate of Confidentiality	Acquired from NIH following IRB approval

Table of Contents

STATEMENT OF COMPLIANCE	1
1 PROTOCOL SUMMARY	1
1.1 Synopsis	1
1.2 Schema	4
1.2.1 Schedule of Activities (SoA)	1
2 INTRODUCTION	3
2.1 Study Rationale	3
2.2 Background	3
2.3 Risk/Benefit Assessment	6
2.3.1 Known Potential Risks	6
2.3.2 Known Potential Benefits	8
2.3.3 Assessment of Potential Risks and Benefits	8
3 OBJECTIVES AND ENDPOINTS	9
4 STUDY DESIGN	12
4.1 Overall Design	12
4.2 Scientific Rationale for Study Design	13
4.3 Justification for Dose	13
4.4 End of Study Definition	13
5 STUDY POPULATION	13
5.1 Inclusion Criteria	13
5.2 Exclusion Criteria	14
5.3 Screen Failures	15
5.4 Strategies for Recruitment and Retention	15
6 STUDY INTERVENTION	17
6.1 Study Intervention(s) Administration	17
6.1.1 Study Intervention Description	17
6.1.2 Dosing and Administration	18
6.2 Measures to Minimize Bias: Randomization and Blinding	19
6.3 Study Intervention Compliance	19
6.4 Concomitant Therapy	19
7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT	
DISCONTINUATION/WITHDRAWAL	20
7.1 Discontinuation of Study Intervention	20
7.2 Participant Discontinuation/Withdrawal from the Study	20
7.3 Lost to Follow-Up	20
8 STUDY ASSESSMENTS AND PROCEDURES	21
8.1 ELIGIBILITY ASSESSMENTS	21
8.2 Efficacy Assessments	22
8.3 Safety and Other Assessments	23
8.4 Adverse Events and Serious Adverse Events	25
8.4.1 Definition of Adverse Events (AE)	25
8.4.2 Definition of Serious Adverse Events (SAE)	25
8.4.3 Classification of an Adverse Event	26
8.4.4 SAFETY EVENT REPORTING	27
8.4.5 Unanticipated Adverse Device Effect Reporting	27
8.4.6 Reporting Events to Participants	27

8.4.7	Reporting of Pregnancy (for healthy control participants).....	28
8.5	Unanticipated Problems	28
8.5.1	Definition of Unanticipated Problems (UP).....	28
8.5.2	Unanticipated Problem Reporting.....	28
8.5.3	Reporting Unanticipated Problems to Participants	29
9	STATISTICAL CONSIDERATIONS	29
9.1	Statistical Hypotheses.....	29
9.2	Sample Size Determination	29
9.3	Populations for Analyses.....	29
9.4	Statistical Analyses.....	29
9.4.1	General Approach.....	29
9.4.2	Analysis of the Primary Endpoint(s).....	30
9.4.3	Analysis of the Secondary Endpoint(s).....	30
9.4.4	Safety Analyses	30
9.4.5	Baseline Descriptive Statistics.....	31
9.4.6	Planned Interim Analyses	31
9.4.7	Tabulation of Individual participant Data	31
9.4.8	Exploratory Analyses.....	31
10	SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS	31
10.1	Regulatory, Ethical, and Study Oversight Considerations	31
10.1.1	Informed Consent Process	31
10.1.2	Study Discontinuation and Closure	32
10.1.3	Confidentiality and Privacy	33
10.1.4	Future Use of Stored Specimens and Data.....	33
10.1.5	Key Roles and Study Governance	34
10.1.6	Safety Oversight	34
10.1.7	Clinical Monitoring	34
10.1.8	Quality Assurance and Quality Control	34
10.1.9	Data Handling and Record Keeping	35
10.1.10	Protocol Deviations	35
10.1.11	Publication and Data Sharing Policy	36
10.1.12	Conflict of Interest Policy.....	36
10.2	Abbreviations	36
10.3	Protocol Amendment History.....	38
11	REFERENCES.....	40
12	Appendix	44
12.1	Mania Assessment questions	44
12.2	AE Check questions	44
12.3	Birth outcomes	44

STATEMENT OF COMPLIANCE

The trial will be conducted in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP), applicable United States (US) Code of Federal Regulations (CFR), and the Brain Behavior Research Foundation Distinguished Investigator Award Terms and Conditions. The Principal Investigator will assure that no deviation from, or changes to the protocol will take place without prior agreement from the Investigational New Drug (IND) or Investigational Device Exemption (IDE) sponsor, funding agency and documented approval from the Institutional Review Board (IRB), except where necessary to eliminate an immediate hazard(s) to the trial participants. All personnel involved in the conduct of this study 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 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. 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: Pilot study using endogenous aperiodic brain activity to personalize transcranial alternating current stimulation as a treatment for antenatal depression: PandA-tACS

Study Description: The purpose of this study is an initial pilot study to develop the safety, feasibility, and tolerability of a novel, individualized transcranial alternating current stimulation (tACS) approach to treat antenatal depression, which delivers personalized stimulation waveforms based on the aperiodic component of individual participant EEG activity to increase excitation in the brain ('PandA-tACS'). HD-EEG is measured from each participant at Baseline, then is computationally modified to mimic the restoration of excitation/inhibition balance seen following rapid-mood shifts during esketamine treatment in depression, and then delivered as a non-invasive brain stimulation treatment. In Phase I, five healthy controls will receive five consecutive days of PandA-tACS. High-density electroencephalography will be collected at Baseline, D1 and D5, as well as intermittently throughout stimulation sessions. Symptoms will be checked at FUP1 (two weeks following intervention). In Phase II, PandA-tACS will be expanded into five patients with antenatal depression with the same study design. In addition to HD-EEG measurements, depression and related symptoms will be measured at D1, D5 in these patients. Depression and related symptoms will be measured again FUP1 (two weeks following intervention) and FUP2 (4-6 weeks post delivery). Birth outcomes will be recorded within 90 days of expected delivery date as an additional form of safety monitoring.

Objectives:

Phase I – Healthy controls

Primary Objectives

1. Investigate safety via the presence of any serious AEs related to stimulation
2. Investigate feasibility of five days of PandA-tACS in healthy controls
3. Investigate tolerability of five days of PandA-tACS in healthy controls

Exploratory Objectives

4. Investigate aperiodic EEG changes following five days of aperiodic tACS in healthy controls

Phase II – Antenatal depression

Primary Objectives

5. Investigate the safety of five days of PandA-tACS in antenatal depression
6. Investigate feasibility of five days of PandA-tACS in antenatal depression
7. Investigate tolerability of five days of PandA-tACS in antenatal depression

Exploratory Objectives

8. Investigate aperiodic EEG changes following PandA-tACS in antenatal depression
9. Investigate changes in depression symptoms two weeks following PandA-tACS in antenatal depression
10. Investigate changes in depression symptoms at 4-6 weeks post-birth (postnatal period)

Endpoints: Phase I – Healthy Controls

Primary Endpoints

- The number of serious adverse events reported in healthy control group [Timeframe: D1 to D5]
- The number of participants enrolled relative to the target recruitment of five participants in healthy control group over the specified 12-month period
- The number of participants who complete all study visits within the intervention and follow-up period in the healthy control group [Timeframe: Baseline to FUP1]
- The number of participants who rate stimulation-related sensations as 'high' within the intervention period in the healthy control group [Timeframe: D1 to D5]
- The proportion of participants who are unable to tolerate stimulation within the intervention period in the healthy control group [Timeframe: D1 to D5]

Exploratory Endpoints

- The change in aperiodic exponent from resting state EEG recordings on the first day and the final day of stimulation in healthy control population. [Timeframe: Baseline to D5]

Phase II – Antenatal Depression

Primary Endpoints

- The number of serious adverse events reported in antenatal depression group [Timeframe: D1 to D5]
- The number of adverse birth outcomes in participants with antenatal depression. Review will consider length of pregnancy in days, delivery (spontaneous, induced, cesarian (planned or acute), vaginal birth, forceps and/or vacuum extraction), gestational age at birth, Apgar 5 min, malformation, child weight at birth in grams, birth size (small for gestational age, appropriate for gestational age, large for gestational age), neonatal need for intensive care, stillbirth, maternal length of stay in hospital, maternal need for intensive care, pre-eclampsia, as well as EPDS score between 2-4 weeks post-delivery [Timeframe: D1 to end of monitoring – within 90 days of expected delivery date]
- The number of participants enrolled relative to the target recruitment of five participants in antenatal depression group over the specified 12-month period
- The number of participants who complete all study visits within the intervention and follow-up period in the antenatal depression group [Timeframe: Baseline to D5]
- The number of participants who rate stimulation-related sensations as 'high' within the intervention period in the antenatal depression group [Timeframe: D1 to D5]
- The proportion of participants who are unable to tolerate stimulation within the intervention period in the antenatal depression group [Timeframe: D1 to D5]

Exploratory Endpoints

- The change in aperiodic exponent from resting state EEG recordings on the first day and the final day of stimulation in antenatal depression population [Timeframe: D1 to D5]
- Change in HDRS-17 score following intervention in the antenatal depression population [Timeframe: D1 to D5, D1 to FUP1, D5 to FUP1]
- Change in Edinburgh Postnatal Depression Scale (EPDS) in the antenatal depression population [Timeframe: D1 to D5, D1 to FUP1, D5 to FUP1]
- Change in Snaith-Hamilton Pleasure Scale (SHAPS) in the antenatal depression population [Timeframe: D1 to D5, D1 to FUP1, D5 to FUP1]
- Change in Clinical Global Impression Scale (CGI) in the antenatal depression population [Timeframe: D1 to D5, D1 to FUP1, D5 to FUP1]
- Change in Quality of Life Enjoyment and Satisfaction Questionnaire (Q-LES-Q-SF) in the antenatal depression population [Timeframe: D1 to D5, D1 to FUP1, D5 to FUP1]

- Change in HDRS-17 score following intervention in the antenatal depression population [Timeframe: FUP1 to FUP2]
- Change in Edinburgh Postnatal Depression Scale (EPDS) in the antenatal depression population [Timeframe: FUP1 to FUP2]
- Change in Snaith-Hamilton Pleasure Scale (SHAPS) in the antenatal depression population [Timeframe: FUP1 to FUP2]
- Change in Clinical Global Impression Scale (CGI) in the antenatal depression population [Timeframe: FUP1 to FUP2]
- Change in Quality of Life Enjoyment and Satisfaction Questionnaire (Q-LES-Q-SF) in the antenatal depression population [Timeframe: FUP1 to FUP2]

Study

Population:

Phase I:

- n=5 female controls
- 18-45 years of age
- No history of depression
- Low suicide risk

Phase II:

- n=5 female antenatal depression
- 18-45 years of age
- 14-32 weeks of pregnancy
- Unipolar, non-psychotic MDD
- HDRS-17 >8
- Low suicide risk

Description of Sites/Facilities

This is a single-site study performed at The University of North Carolina at Chapel Hill.

Enrolling

Participants:

Description of Study Intervention:

PandA-tACS is a form of tACS which uses personalized, naturalistic tACS waveforms based on aperiodic features in individual participant brain activity which has been computationally modified to non-pharmacologically restore excitation/inhibition balance.

Study

Duration:

15 months

Participant Duration:

Healthy controls (Phase I): Fourteen hours across seven visits over four weeks.
Antenatal depression (Phase II): Sixteen hours across eight visits over 15-33 weeks (depending on gestational age at treatment)

1.2 SCHEMA

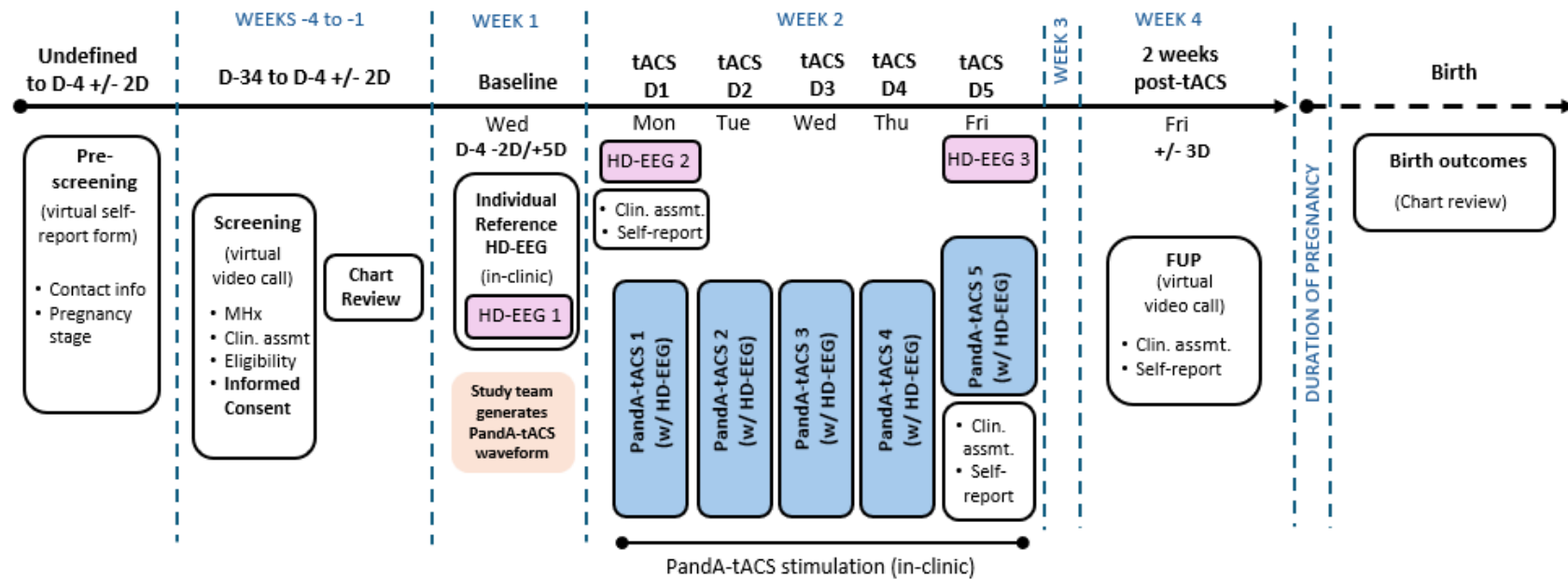


Figure 1: Schema of PandA-tACS intervention.

Schema represents study activities for Phase II antenatal depression population. Phase I healthy control population will undertake all activities except clinical-related activities and measures including pregnancy stage, medical history, symptom-related clinical assessments, FUP2, and pregnancy outcome review. Phase I healthy control population will be screened using the DIAMOND Self Report Screener.

MDD, major depressive disorder; MHx, medical history; Clin. assmt., clinical assessment; HD-EEG, high-density electroencephalography; PandA-tACS, Personalized Antenatal Depression Aperiodic Transcranial Alternating Current Stimulation; FUP, follow-up

1.2.1 SCHEDULE OF ACTIVITIES (SOA)

	Pre-screening Undefined to D-1 (virtual)	Chart review*	Screening D-30 to D-1 (virtual)	Baseline Wed -2D/+5D (in-clinic)	PandA-tACS D1 Mon (in-clinic)	PandA-tACS D2 Tue (in-clinic)	PandA-tACS D3 Wed (in-clinic)	PandA-tACS D4 Thu (in-clinic)	PandA-tACS D5 Fri (in-clinic)	FUP 1 2 weeks-post D5 Fri +/-3D (virtual)	FUP 2* 4-6 weeks post delivery	Pregnancy outcomes (within 90 days of expected delivery date)
Procedures												
Waived consent	X											
Pre-screening form	X											
Contact information	X											
Pregnancy stage	X*	X*										
HIPAA Waiver	X*											
Verbal consent			X									
Informed consent			X									
HIPAA Authorization			X ⁵									
Medical history		X*	X									
Obtain OB information			X*									
Eligibility Chart Review		X*										
Concomitant medication and therapy		X*	X	X	X	X	X	X	X	X	X*	
DIAMOND self-report screener	X											
DIAMOND (full)			X ⁶									
Maudsley Staging Method*			X*									
Urine pregnancy test				X ⁴								
HDRS-17			X*		X*				X*	X*	X*	
EPDS					X*				X*	X*	X*	
C-SSRS			X		X		X		X	X	X*	
Mania Assessment			X		X		X		X	X	X*	
YMRS ¹			X ¹		X ¹				X ¹	X ¹	X ^{*1}	
SHAPS					X*				X*	X*	X*	
Q-LES-Q-SF					X*				X*	X*	X*	
CGI					X*				X*	X*	X*	
Eligibility review		X*	X	X	X							
W-9 Form/W-8 BEN				X								
Demographics				X								
HD-EEG				X	X ³	X ³	X ³	X ³	X			
Generate PandA-tACS waveform				X								
Blood pressure monitoring ⁷				X	X	X	X	X	X			
Likert Scales				X	X	X	X	X	X	X	X*	
Prenatal check (bleeding, cramping, loss of fluid, normal fetal movement)					X*	X*	X*	X*	X*	X*		
PandA-tACS					X	X	X	X	X			
Stimulation sensation questionnaire					X	X	X	X	X			
AE check					X	X	X	X	X	X	X*	
AE log ²					X ²	X ²	X ²	X ²	X ²			
Pregnancy and birth outcome chart review												X*

MDD, major depressive disorder; Dx, diagnosis; MHx, medical history; Clin. assmt., clinical assessment; HD-EEG, high-density electroencephalography; PandA-tACS, Personalized Antenatal Depression Aperiodic Transcranial Alternating Current Stimulation; FUP, follow-up; HDRS-17, Hamilton Depression Rating Scale 17 Items; SHAPS, Snaith-Hamilton Pleasure Scale; Q-LES-Q-SF, Quality of Life Enjoyment and Satisfaction Questionnaire Short Form; CGI, Clinical Global Impression Scale; AE, adverse event; YMRS, Young Mania Rating Scale; DIAMOND, Diagnostic Interview for Anxiety, Mood, and OCD and Related Neuropsychiatric Disorders; EPDS, Edinburgh Postnatal Depression Scale; OB, obstetrician

⁺*DIAMOND self-report screener administered for HCs only*

^{*}*Only administered for Phase II (antenatal depression population)*

¹*Only administered if participant answers 'yes' to any question on the Mania Assessment (on any day of Mania Assessment)*

²*Only administered if AE is found*

³*HD-EEG net placed over stimulation electrodes to capture HD-EEG immediately before, after, and in between stimulation blocks*

⁴*Only administered to HC participants*

⁵*All participants will sign the HIPAA authorization. If any participant in the HC group becomes pregnant during the study, we will access that participant's medical records for safety monitoring.*

⁶*Full DIAMOND only administered as needed (according to self report on prescreen)*

⁷*Measured immediately before and after stimulation*

2 INTRODUCTION

2.1 STUDY RATIONALE

Antenatal depression is one of the most under-investigated and under-treated disorders in psychiatry. Antenatal depression has a prevalence of 7-20% among pregnant women and tends to persist or reoccur in subsequent pregnancies (Dadi et al., 2020). Alongside impacts on maternal quality of life, health-seeking behavior, and increased risk behavior such as substance misuse, antenatal depression is also associated with fetal risks including low birth weight, preterm birth, and pregnancy complications (Dadi et al., 2020). Antenatal depression is also related to ongoing sequelae following birth including postpartum depression and psychosis, serious illnesses associated with suicide, infanticide, and homicide (Bowen & Muhajarine, 2006). Despite this high disease burden, treatment options are complex since effective pharmacological treatments require additional considerations relating to the potential fetal impact and negative patient perceptions about use of medications during pregnancy.

Our goal is to develop a safe, non-pharmacological approach to induce a rapid improvement in depression symptoms based on our recent findings of how esketamine changes specific features of brain activity measured by EEG (particularly aperiodic activity). We propose a novel, individualized tACS approach to treat antenatal depression which delivers personalized stimulation waveforms to target aperiodic brain activity ('PandA-tACS'). Here, brain activity from each participant is used to generate an individualized stimulation waveform, which is delivered as a non-invasive neurostimulation treatment to restore excitation/inhibition balance in the brain, with the goal of generating a rapid improvement in depression symptoms akin to esketamine. This approach is motivated by our work on tACS over the last 15 years that demonstrates how mimicking endogenous brain activity increases efficacy in modulating brain activity (Alexander et al., 2019; Fröhlich & McCormick, 2010; Huang et al., 2021; Schwippel et al., 2024). Rather than using artificial rhythmic stimulation to mimic brain activity, we will deliver a waveform built on measured endogenous brain activity and altered using rational design to achieve desired changes in network dynamics. We hypothesize that PandA-tACS will be safe, feasible, and tolerable.

2.2 BACKGROUND

As one of the most under-investigated and under-treated disorders, antenatal depression is a significant concern in psychiatry. Despite the high disease burden associated with antenatal depression, treatment options are complex to navigate since effective pharmacological treatments require additional considerations relating to fetal impacts and patient desire to avoid medication during pregnancy (Freeman, 2007). In 2019, a rapid-acting, effective antidepressant, esketamine, was approved by the US-FDA for the management of treatment-resistant depression. Compared to therapy, where noticeable effects generally take several weeks, esketamine is associated with rapid improvements on the scale of hours or days (Hock et al., 2022). However, access to esketamine and other antidepressants in antenatal depression is challenging due to high potential for abuse and potential effects on cognitive function (Freeman, 2007). Further, patient acceptability remains low for antidepressants in general due to uncertainty regarding fetal effects (Battle et al., 2013). The increased risk of antenatal depression on both maternal mental health and childhood development along with the scarce pharmacological treatment options amplifies the need for effective non-pharmacological treatments in this underserved population.

Currently, the leading non-pharmacological treatment for antenatal depression is electroconvulsive therapy (ECT). The New York State Office of Mental Health (.gov) guidelines state *"Pregnant women and nursing mothers may receive ECT during all trimesters of pregnancy, puerperium and nursing. ECT may be less risky than alternate pharmacologic treatment or non-treatment of mental illness during pregnancy."* A

review from Australia found that 14% of women with depression during pregnancy were prescribed ECT between 2017 and 2019 (Saluja et al., 2024). Further, a systematic review of 339 cases internationally between 1941 to 2007 found that ECT was used across all three trimesters of pregnancy, with a mean number of 11 treatments per patient. This systematic review found a rate of at least partial response to be 84%, alongside a low risk of adverse events; concluding that ECT should be strongly considered in women with severe depression during pregnancy (Anderson & Reti, 2009). A more recent review of the last 50 years of ECT trials reported that there are no associations between ECT and congenital anomalies, neurocognitive disturbances, nor miscarriage, where pregnancy does not convey any additional risk in ECT (Ward et al., 2018). However, ECT is typically reserved for severe treatment-refractory patients, limiting its accessibility to the general antenatal depression population.

Low-intensity forms of non-invasive brain stimulation techniques, such as transcranial alternating current stimulation (tACS) or transcranial magnetic stimulation (TMS) represent another potentially promising, yet generally unexplored, non-pharmacological treatment alternative which could be used across the range of antenatal depression severity and stage of pregnancy. These techniques are advantageous over ECT in the sense that they require less specialist expertise to administer the treatment, have increased portability, and do not have a need for anesthesia, therefore increasing accessibility compared to ECT. TMS (Nahas et al., 1999) has been used off-label as a treatment for depression during pregnancy. Several pilot studies support efficacy and safety, including a review of ten reports of 67 births over 20 years where TMS was used in all trimesters, finding no serious events for any mother or baby (Konstantinou et al., 2020; Pridmore et al., 2021). However, a lack of patient education about the technique contributes to lower acceptability for TMS (Kim et al., 2011). Recently, rhythmic, low-intensity electric stimulation, transcranial alternating current stimulation (tACS), has emerged as a promising treatment approach for depression (Alexander et al., 2019; Fröhlich & McCormick, 2010; Schwippel et al., 2024). tACS delivers currents which are up to 900 times less intense than ECT, and 100 times less intense than TMS. Further, it is associated with a lower side effect profile compared to both TMS and widely-used transcranial direct current stimulation (tDCS), including no history of seizure risk, and an association with generally only mild and transient side effects such as tingling and itching (Antal et al., 2017). Further, tACS has increased portability compared to TMS, allowing future at-home usage (Antal et al., 2017). Overall, this suggests that tACS may be a safe, effective, and accessible treatment for antenatal depression.

Despite a great amount of potential utility alongside a high level of safety, clinical trials of both TMS and tACS have traditionally excluded pregnant people, despite the fact that there is no evidence to support that neuromodulation is unsafe in this population (Konstantinou et al., 2020), meaning that the efficacy of tACS in antenatal depression remains unknown. This has led to a lack of treatment development for antenatal depression, leaving this vulnerable population underserved. Two independent reviews conducted in 2015 and 2020 supported the use of neuromodulation in antenatal depression, concluding that the potential adverse fetal effects of non-invasive brain stimulation during pregnancy are mostly theoretical, and outweigh the risk of untreated antenatal depression or the risks of antidepressant medication during pregnancy (Konstantinou et al., 2020).

The evidence for the efficacy of neuromodulation for depression has grown in recent years following application of personalized stimulation protocols. Previous studies have indicated that tACS is especially effective when delivered in an individualized manner; that is, when stimulation parameters are adjusted for each patient based on individual brain activity (Fröhlich & McCormick, 2010; Schwippel et al., 2024). In the past, individualization has been based on rhythmic (periodic) brain activity features (e.g. ‘brain waves’) (Schwippel et al., 2024), however, non-rhythmic (aperiodic) activity, which reflects general excitation and inhibition in the brain, has been neglected despite its important role in neuronal dynamics (Donoghue et al., 2020) and the demonstration of entrainment efficacy at extremely low amplitude of naturalistic electric fields in a preclinical model (Fröhlich & McCormick, 2010).

Our goal with this proposal is to develop a safe, non-pharmacological approach using a novel method of non-invasive brain stimulation personalized to aperiodic brain activity features to induce a rapid improvement in symptoms. Importantly, this approach adheres to the standard tACS procedures relating to electrode preparation, electrode application, current amplitude, and delivery with a standard tACS device.

Our proposed approach is based on our recent findings of how esketamine changes specific features of brain activity measured by EEG. Recently, our research team completed a trial which used high-density electroencephalography (HD-EEG) to examine changes in brain activity, particularly relating to aperiodic features, over a 90-minute period following nasal esketamine administration in eight individuals with major depressive disorder. Aperiodic activity is of particular interest in depression, as it has been linked to an excitation/inhibition balance shift in the brain, leaning towards increased inhibition, which is characterized by a steeper aperiodic exponent (slope) (Donoghue et al., 2020). Results from this trial demonstrated a significant reduction in the aperiodic exponent (i.e., a 'flatter' exponent) following esketamine administration which was most prominent in left central-frontal electrodes, indicating an excitation/inhibition shift towards excitation (i.e., towards restoring normal balance) (Fig. 1, unpublished data). Further, this change in exponent was associated with increased self-ratings of 'highness', suggesting a potential effect of esketamine on mood alongside brain activity changes. These results align with previous work using intracranial recordings, which found that a flatter slope (indicating greater excitation) was associated with a more euthymic mood state in depressed patients (Hacker et al., 2023) and support the theory that improved depression symptoms are associated with increased excitation.

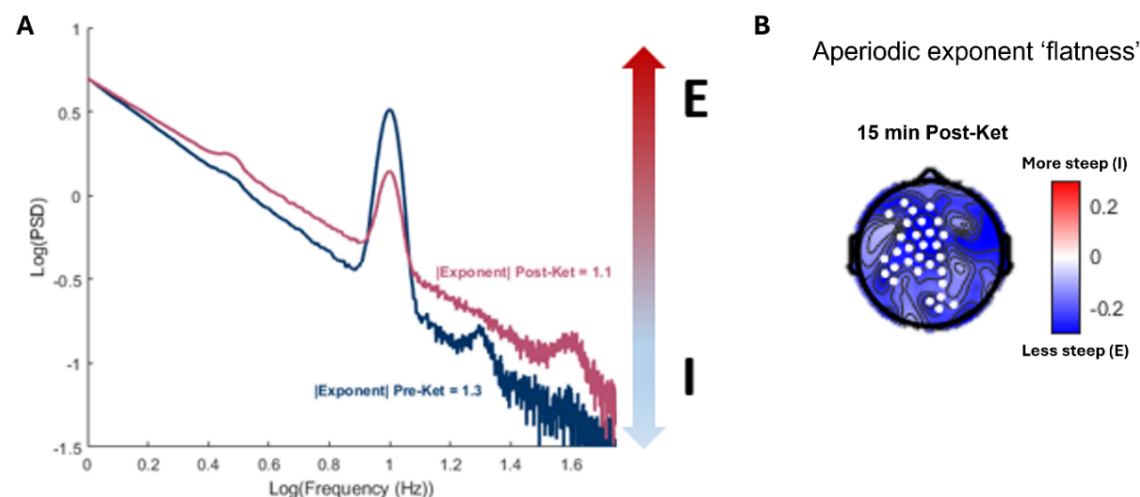


Figure 1. Model of excitatory /inhibitory balance based on the aperiodic exponent.
A: Log-transformed spectrum data of average aperiodic exponents in depressed patients before esketamine administration (blue), and after esketamine administration (red), demonstrating a flatter exponent following esketamine (greater excitation). B: Topographic map of aperiodic exponent localized to left fronto-central regions. E, excitation; I, inhibition.

We use the results from this study to inform the parameters for a novel individualized form of neuromodulation, known as 'PandA-tACS' (Personalized Antenatal Depression Aperiodic Transcranial Alternating Current Stimulation), which targets aperiodic exhibition/inhibition balance in antenatal depression. Here, brain activity from each participant will be used to generate individualized stimulation waveforms that are subsequently modified to shift excitatory/inhibitory balance. This approach is motivated by our work on tACS over the last 15 years that demonstrates how mimicking endogenous brain activity increases efficacy in modulating brain activity (Alexander et al., 2019; Fröhlich & McCormick, 2010; Huang et al., 2021; Schwippel et al., 2024). Rather than using artificial rhythmic stimulation to mimic

endogenous brain activity, we will deliver a waveform which closely replicates endogenous brain activity, with small adjustments to restore excitatory/inhibitory balance, while maintaining the same safety parameters used in normal tACS setup (e.g., a tACS device with safety cutoffs, standard electrode preparation and application, and rigorous safety monitoring). In this trial, we hope to establish the safety, feasibility, tolerability and preliminary efficacy of PandA-tACS, first in healthy controls, followed by an antenatal depression population. If successful, this project will inform a larger clinical trial to further develop this technique as potential future treatment.

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. To avoid breaches in confidentiality, study documents that contain personal information, including the informed consent document, and the document that links study ID numbers to personal identifying information are kept in locked filing cabinets in locked rooms, separate from any source documents containing participant dummy identifiers, or stored in password protected spreadsheets only accessible to study personnel. Only the audio of the screening on the UNC HIPAA-compliant Zoom recording will be saved and the video will be discarded. Any paper data is stored in locked cabinets inside locked offices; electronic data will be stored only on password-protected computers. Questionnaires will be collected electronically through REDCap. De-identified data are stored in a UNC-approved secure project SharePoint site. Interviews will be conducted over the HIPAA approved Zoom account at UNC. Only study personnel will have access to the data. All study staff participate in annual human participant training that includes education about responsibilities to minimize risk of confidentiality breach.

Risk of Embarrassment: Self-report assessments contain questions regarding sensitive personal information, which may lead to feelings of embarrassment. This risk is necessary in order to assess mood symptoms and associated psychopathology. Participants will be assured upon intake that only study personnel will see any clinical ratings and that self-identifying information will not be collected alongside HIPAA protected information. Further, healthy control participants will be required to take a pregnancy test at Baseline. The study staff member will inform the participant of the pregnancy test outcome. If a participant is unexpectedly found to be pregnant, it may cause embarrassment and/or distress. Participants will be informed at screening that they will be required to take a pregnancy test at the baseline session, and that the result will be communicated to them. Patients who are found to be pregnant will be referred to their primary care provider.

Risk of Injury and Discomfort: The side effects of tACS are mild and transient; in fact, low intensity transcranial current stimulation, such as tACS, has been used for several years without any report of serious side effects (Antal et al., 2017). Furthermore, tACS has nothing to do with electroconvulsive therapy that applies stimulation currents of up to 900x greater intensity. Rather, transcranial current stimulation is so weak that it does not cause super-threshold activation of neurons (Fröhlich & McCormick, 2010). However, tACS can produce sensations, such as transient mild to moderate tingling, burning, or itching under the electrode sites. In a previous trial, participants from all three groups of stimulation (active 10Hz, active 40 Hz and sham) reported either absent or mild side effects, and there was no difference between the groups with the exception of “flickering lights” (or phosphenes, $p = 0.014$) (Alexander et al., 2019). Further, in our previous open label trial in depression (Schwippel et al., 2024), participants rated all 12 side effect items after personalized tACS as “absent” or “low” on average. One participant experienced nausea and vomiting

during the first treatment session. These transient symptoms were deemed to be mild and considered unlikely to be related to stimulation by the study clinician since the participant did not disclose an ongoing COVID-19 infection, which included headache, dizziness, and nausea before stimulation onset. No serious adverse events occurred, and no reported AEs significantly outlasted the stimulation treatment typically resolving prior to the patient leaving the clinic. To monitor these symptoms in the current trial, we will be administering a stimulation sensation questionnaire after each stimulation session. Mild to moderate sensation ratings during tACS application are expected (Antal et al., 2017). Reports of strong sensation ratings will be discussed with the study clinician, who will make the determination of whether this represents an untoward medical effect. Adverse events will be captured at each research visit in addition to spontaneous reporting. Research personnel are present during the full experiment.

Risk during pregnancy. There are very few studies which have used neuromodulation during pregnancy. However, there is no evidence to support that neuromodulation is unsafe in this population (Konstantinou et al., 2020). ECT, which is a non-invasive brain stimulation treatment using currents up to 900 times higher than tACS is used widely in severe and/or treatment refractory antenatal depression (Anderson & Reti, 2009). Transcranial magnetic stimulation (TMS) (Nahas et al., 1999) has been used off-label as a treatment for depression during pregnancy. Several pilot studies support efficacy and safety of non-invasive brain stimulation during pregnancy, yet a larger multi-site has not yet been conducted (Konstantinou et al., 2020). Although non-invasive neuromodulation is not known to be associated with safety risks in pregnancy (Konstantinou et al., 2020), we will record birth outcomes within 90 days of birth to monitor: length of pregnancy in days, delivery (spontaneous, induced, cesarian (planned or acute), vaginal birth, forceps and/or vacuum extraction), gestational age at birth, Apgar 5 min, malformation, child weight at birth in grams, birth size (small for gestational age, appropriate for gestational age, large for gestational age), neonatal need for intensive care, stillbirth, maternal length of stay in hospital, maternal need for intensive care, pre-eclampsia, as well as EPDS score between 2-4 weeks post-delivery. Further, we will only enroll participants between 14-32 weeks of pregnancy to ensure that the intervention does not interact with critical periods of pregnancy (i.e. in the first weeks following embryo implantation and the final weeks before birth), although ECT is routinely used in all pregnancy trimesters so PandA-tACS could theoretically be safely used throughout the pregnancy.

Risk of worsening, suicidal ideation/attempt: Patients with MDD have an about 20 times higher rate of suicide than the general population. We have no evidence that PandA-tACS will in any way increase this likelihood. Rather, active engagement in a clinical trial may offer hope and structure to the participants. In our previous open-label trial utilizing closed-loop tACS, no participants reported an increase in suicidal ideations following stimulation (Schwippel et al., 2024).

We will be using the Columbia Suicide Severity Rating Scale (C-SSRS, triage form) (Posner et al., 2011) to determine risk for suicide throughout the study. Inclusion criteria state that the participant must be low suicide risk (defined for this study as no active suicidal ideation in the past month and no suicide attempts, preparatory actions, or significant non-suicidal self-harm in the previous 2 years). Participants with risk greater than this at baseline will not be included in this study. In addition, the answers to suicide-related questions on the HDRS-17 (Williams, 1988) will be monitored throughout the study.

If an enrolled participant reports active suicidal ideation (or higher levels of risk) during the course of the study, the participant will be evaluated by a trained clinician, who will determine if an acute assessment by Dr. Riddle, Dr. Feldman, or another trained clinician is necessary. This assessment may include facilitating participant contact 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, the clinician will assist in establishing a care plan. An expedited referral to UNC Psychiatry will be made if needed. 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 first responders to ensure that the participant is safely escorted to the Emergency Department for further care. Dr. Riddle, Dr Feldman, or another trained clinician will decide if participation should be stopped after the acute assessment. If the participant is hospitalized, the intervention will be discontinued but study assessments will continue to be administered where possible for safety purposes.

2.3.2 KNOWN POTENTIAL BENEFITS

There are few individual benefits to participants in this study. Participants in this study may experience some degree of relief from depression symptoms following five days of PandA-tACS given our previous double-blind sham-controlled study demonstrating that five days of open-loop (non-individualized) tACS results in a significant improvement in depression symptoms alongside a decrease in left frontal alpha power in the brain (Alexander et al., 2019), as well as our previous open-label trial of individualized closed-loop tACS showing a similar improvement in depression symptoms which were also correlated with a reduction in alpha power (Schwippel et al., 2024). However, the duration of these effects is not yet clear.

This study will greatly benefit society as a whole by contributing to the development of a novel neuromodulation technique in a vulnerable population with few treatment options.

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 novel, rapid-acting treatments for MDD, the potential benefits outweigh the potential risks. We also note that the FDA has recently confirmed a non-significant risk (NSR) status for a similar tACS trial in MDD.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
<i>Phase I – Healthy controls</i>		
Investigate short term safety of five days of Panda-tACS in healthy controls	The number of serious adverse events reported in healthy control group [Timeframe: D1 to D5]	Serious AEs will be used as a measure of safety during the stimulation period.
Investigate feasibility of five days of Panda-tACS in healthy controls	The number of participants enrolled relative to the target recruitment of five participants in healthy control group over the specified 12-month period	These endpoints are designed to test the feasibility of the intervention.
	The number of participants who complete all study visits within the intervention and follow-up period in the healthy control group [Timeframe: Baseline to FUP1]	
Investigate tolerability of five days of Panda-tACS in healthy controls	The number of participants who rate stimulation-related sensations as 'high' within the intervention period in the healthy control group [Timeframe: D1 to D5]	These endpoints will provide information about tolerability.
	The proportion of participants who are unable to tolerate stimulation within the intervention period in the healthy control group [Timeframe: D1 to D5]	
<i>Phase II – Antenatal depression</i>		
Investigate the safety of five days of Panda-tACS in antenatal depression	The number of serious adverse events reported in antenatal depression group [Timeframe: D1 to D5]	Serious AEs will be used as a measure of safety during the stimulation period.
	The number of adverse birth outcomes in participants with antenatal depression. Review will consider length of pregnancy in days, delivery (spontaneous, induced, cesarian (planned or acute), vaginal birth, forceps and/or vacuum extraction), gestational age at birth, Apgar 5 min, malformation, child weight at birth in grams, birth size (small for gestational age, appropriate for gestational age, large for gestational age), neonatal need for intensive care, stillbirth, maternal length of stay in hospital, maternal need for intensive care, pre-eclampsia, as well as EPDS score between 2-4 weeks post-delivery [Timeframe: D1 to end of monitoring – within 90 days of expected delivery date]	While Panda-tACS is not expected to result in any adverse events related to pregnancy, these endpoints will allow quantification of any adverse effects to the birth giver or baby.

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Investigate feasibility of five days of PandA-tACS in antenatal depression	- The number of participants enrolled relative to the target recruitment of five participants in antenatal depression group over the specified 12-month period - The number of participants who complete all study visits within the intervention period in the antenatal depression group [Timeframe: Baseline to D5]	These endpoints are designed to test the feasibility of the intervention.
Investigate tolerability of five days of PandA-tACS in antenatal depression	- The number of participants who rate stimulation-related sensations as 'high' within the intervention period in the antenatal depression group [Timeframe: D1 to D5] - The proportion of participants who are unable to tolerate stimulation within the intervention period in the antenatal depression group [Timeframe: D1 to D5]	These endpoints will provide information about tolerability.
Exploratory		
<u>Phase I – Healthy controls</u>		
Investigate aperiodic EEG changes following five days of aperiodic tACS in healthy controls	The change in aperiodic exponent from resting state EEG recordings on the first day and the final day of stimulation in healthy control population. [Timeframe: Baseline to D5]	As this method of tACS personalization involves changing the aperiodic element of the EEG, it is hypothesized that aperiodic activity in the participant's brain activity following stimulation would change.
<u>Phase II – Antenatal depression</u>		
Investigate aperiodic EEG changes following five days of aperiodic tACS in antenatal depression	The change in aperiodic exponent from resting state EEG recordings on the first day and the final day of stimulation in antenatal depression population [Timeframe: D1 to D5]	As this method of tACS personalization involves changing the aperiodic element of the EEG, it is hypothesized that aperiodic activity in the participant's brain activity following stimulation would change.
Investigate changes in depression symptoms following five days of aperiodic tACS in antenatal depression	Change in HDRS-17 score following intervention in the antenatal depression population [Timeframe: D1 to D5, D1 to FUP1, D5 to FUP1]	This will provide information for whether the treatment is clinically effective. HDRS-17 is the standard clinician-

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
	• Change in Edinburgh Postnatal Depression Scale (EPDS) in the antenatal depression population [Timeframe: D1 to D5, D1 to FUP1, D5 to FUP1] • Change in Snaith-Hamilton Pleasure Scale (SHAPS) in the antenatal depression population [Timeframe: D1 to D5, D1 to FUP1, D5 to FUP1] • Change in Clinical Global Impression Scale (CGI) in the antenatal depression population [Timeframe: D1 to D5, D1 to FUP1, D5 to FUP1] • Change in Quality of Life Enjoyment and Satisfaction Questionnaire (Q-LES-Q-SF) in the antenatal depression population [Timeframe: D1 to D5, D1 to FUP1, D5 to FUP1]	administered instrument to measure change in symptom severity in people with MDD.
Investigate changes in depression symptoms following five days of aperiodic tACS in postnatal depression	• Change in HDRS-17 score following intervention in the antenatal depression population [Timeframe: FUP1 to FUP2] • Change in Edinburgh Postnatal Depression Scale (EPDS) in the antenatal depression population [Timeframe: FUP1 to FUP2] • Change in Snaith-Hamilton Pleasure Scale (SHAPS) in the antenatal depression population [Timeframe: FUP1 to FUP2] • Change in Clinical Global Impression Scale (CGI) in the antenatal depression population [Timeframe: FUP1 to FUP2] • Change in Quality of Life Enjoyment and Satisfaction Questionnaire (Q-LES-Q-SF) in the antenatal depression population [Timeframe: FUP1 to FUP2]	This will provide information for whether any treatment effects persist at the post-natal follow-up.

4 STUDY DESIGN

4.1 OVERALL DESIGN

This study is a single site, open-label pilot study involving five consecutive days of PandA-tACS, and consisting of two phases, involving application first in healthy controls, followed by application in an antenatal depression population. The purpose of this study is to develop novel, individualized transcranial alternating current stimulation (tACS) approach, which delivers personalized stimulation waveforms to target aperiodic brain activity as a treatment for antenatal depression ('PandA-tACS'). Here, brain activity from each participant is used to generate an individualized stimulation waveform, which is computationally modified to non-pharmacologically restore excitation/inhibition balance, then delivered as a tACS treatment. This study will be conducted in two Phases, including Phase I in healthy controls to test safety, feasibility and efficacy, followed by Phase II in antenatal depression patients

Following the pre-screen (online questionnaire) and virtual screening (video interview with clinician, where electronic informed consent will be given), participants will first attend the clinic on a Wednesday (-2/+5 days) for the Baseline session (i.e., this session can also occur on Stimulation D1), where a full high-density EEG (HD-EEG) will be collected. A personalized tACS treatment waveform will be generated from the participant's HD-EEG, involving computational alteration of the EEG signal to increase the excitation balance. This altered activity will then be used to generate a stimulation waveform which mimics natural, endogenous brain activity. Participants will return to the clinic from Monday to Friday in the following week for five consecutive days of PandA-tACS for 40-50 minutes each day. PandA-tACS will be applied through three electrodes (two on the prefrontal cortex in phase, and one on the midline (out of phase), where a maximum of 2mA zero-to-peak will be delivered. On each stimulation day, a HD-EEG net will be placed over the top of the stimulation electrodes to record brain activity immediately before, after, and throughout the stimulation period. On D1 (Monday) and D5 (Friday), a full HD-EEG will occur to facilitate measurement of brain activity changes following the intervention. In the antenatal depression population, depression symptoms will be measured at D1, D5, at a two-week virtual follow-up (FUP1), and at 4-6 weeks following birth (virtual; FUP2), and birth outcomes will be recorded within 90 days of birth as an additional form of safety monitoring (see Appendix 12.3).

All participants must be at a low risk of suicide (defined for this study as no active suicidal ideation in the past month and no suicide attempts, preparatory actions, or significant non-suicidal self-harm in the previous 2 years) as assessed by the Columbia Suicide Severity Rating Scale (C-SSRS). Healthy control participants will be females aged 18-45 years. All antenatal depression participants will be females aged 18-45 years and will be between 14-32 weeks of pregnancy. Only females are included in this study to avoid confounding effects of non-biological hormones on outcomes. For the antenatal depression population, participants must have unipolar, non-psychotic MDD, with a HDRS-17 > 8. These participants will be outpatients and may or may not seek or receive concurrent mental health care from a family practitioner, therapist or psychiatrist.

Additionally, for antenatal depression participants, a medical chart review will be conducted following the prescreen (prior to the remote screening visit) to assess eligibility. Here, participants will be asked to consent to the medical chart review on the prescreen (HIPAA Waiver). If they consent, the study team will access the medical record to obtain medical information relevant to inclusion/exclusion criteria. If the participant is deemed eligible from the medical record review, they will be scheduled for a screening session.

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

This study is open-label, with safety, feasibility, and tolerability as primary outcomes. It is vital to conduct this study in an open-label study design with these outcomes as it involves a novel form of personalized tACS in a vulnerable population. Data from this study will inform a larger scale efficacy trial.

4.3 JUSTIFICATION FOR DOSE

PandA-tACS is designed to mimic individualized, naturalistic brain activity, which is hypothesized to increase efficacy of treatment by enhancing target engagement through improved entrainment of natural brain activity. The duration of the trial (five consecutive days for 40 minutes per day), location of stimulation (three electrode montage), and intensity of stimulation (max 2mA) are informed by previous trials in our research group using tACS as a treatment for depression (Alexander et al., 2019; Schwippel et al., 2024).

4.4 END OF STUDY DEFINITION

A participant is considered to have completed the study if they have completed all phases of the study including the last visit or the last scheduled procedure shown in the Schedule of Activities (SoA), Section 1.3.

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

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

1. Female aged 18 - 45
- Capacity to understand all relevant risks and potential benefits of the study as determined by study staff (provision of informed consent)
- Stated willingness to comply with all study procedures and availability for the duration of the study
2. Low suicide risk (defined for this study as no active suicidal ideation in the past month and no suicide attempts, preparatory actions, or significant non-suicidal self-harm in the previous 2 years). Risk will be assessed utilizing the C-SSRS screen and triage version with further exploration of positive responses.

For healthy control population:

3. Use of highly effective contraception for at least 1 month prior to screening and agreement to use such a method during study participation, according to NIH Therapeutics Research Program Guidelines

Additional for antenatal depression population:

4. Between weeks 14-32 of viable singleton pregnancy
5. Established obstetric care through UNC
6. Currently meet DSM-5 criteria of unipolar, non-psychotic MDD which is confirmed by the DIAMOND
7. HDRS-17 score >8

5.2 EXCLUSION CRITERIA

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

1. DSM-5 diagnosis of severe alcohol use disorder (AUD) within the last 12 months, as evidenced by the DIAMOND
2. DSM-5 diagnosis of moderate to severe substance use disorder (excluding tobacco) within the last 12 months, as evidenced by the DIAMOND
3. Lifetime history of bipolar disorder, as evidenced by DIAMOND
4. Schizophrenia spectrum and other psychotic disorders, as evidenced by DIAMOND
5. History of autism spectrum disorder
6. Initiated any new psychotropic medication in the 6 weeks prior to screening or had a dose change in the preceding 6 weeks
7. Initiated a new course of psychotherapy in the 6 weeks preceding screening
8. Received any neurostimulation treatment in the 6 weeks preceding screening
9. History of seizures (excluding febrile seizures in childhood or Electroconvulsive Therapy (ECT) induced seizures)
10. Neurological disorders that would increase risk of participation or present a significant confounder in the opinion of the investigator (for example, dementia, history of stroke, Parkinson's disease, multiple sclerosis, history of traumatic brain injury with prolonged loss of consciousness, ruptured cerebral aneurysm, previous CNS radiation)
11. Previously failed to respond to ECT or transcranial magnetic stimulation (TMS)
12. Prior brain surgery and/or brain implants
13. Implanted medical device that uses electricity
14. Currently enrolled in another clinical trial for depression
15. Unstable medical disorder or anything that would place the participant at increased risk or preclude the participant's full compliance with or completion of the study, in the opinion of the Investigator

Additional for the healthy control population:

16. Current pregnancy or lactation (as determined by urine pregnancy test)
17. History of depression, as evidenced by DIAMOND

Additional for the antenatal depression population:

18. History of any of the following conditions:
 - Diabetes (gestational or general history)
 - Pre-term delivery (<37 weeks)
 - Eclampsia
 - Pre-eclampsia with severe features
 - Asthma requiring daily medication
 - Chronic hypertension
 - Immune thrombocytopenia (ITP)
 - Hyperthyroidism requiring medication
 - Pre-pregnancy BMI 40 or more
 - In vitro fertilization (IVF)
 - Mullerian anomaly of uterus
 - Organ transplant
 - Prior history of deep vein thrombosis/pulmonary embolism (DVT/PE) or plan for anticoagulation during pregnancy
 - Fetus with autoimmune hydrops
 - Abnormal placenta
19. Current pregnancy:

- HIV/Hep B/Hep C with detectable viral loads
- Anemia [Hemoglobin under 11.0] upon entry to prenatal care
- No scheduled prenatal visits by 15 weeks
- Placenta previa
- Placenta accreta spectrum (PAS)
- Pre-eclampsia
- Gestational diabetes
- Gestational hypertension
- Fetus with abnormal chromosomes
- Cervical length < 2.5 cm
- Presence of cerclage or vaginal progesterone to decrease chance of pre-term labor
- Fetal growth restriction
- Macrosomia
- Polyhydramnios
- Oligohydramnios
- Rupture of membranes
- Hyperemesis Gravidarum (HEG)
- Confirmation testing for Tri 13/18/21
- Congenital anomalies on anatomy ultrasound that do not resolve with follow-up ultrasound

20. Other cause of markedly high-risk pregnancy as determined by the Investigator

5.3 SCREEN FAILURES

A screen failure will be defined as someone who appears at and participates in the virtual screening session but does not meet inclusion/exclusion criteria for entry into the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in this trial (screen failure) during their initial screening cannot be re-evaluated for entry into the study later if deemed appropriate by the Investigator, unless the reason for ineligibility relates to a recent change in medication, therapy or neurostimulation treatment, or a gestational age which is below the minimum cutoff, in which case the participant may be re-screened once the required amount of time has passed since the change according to inclusion/exclusion criteria. The subject's exclusion from the study, and reason for ineligibility, will be documented in the Screening & Enrollment Log.

Participants who are found to be ineligible at screening can be provided with the Mental Health Resource sheet for further information.

5.4 STRATEGIES FOR RECRUITMENT AND RETENTION

The target sample size is five healthy controls and five females with antenatal depression over the course of a 12-month period (recruitment rate of just under one participant per month). This clinical trial will utilize multiple recruitment strategies to communicate this opportunity to as many potential participants as possible. The first means of recruitment is through a referral process. Participants can be referred to the study through their primary mental health care provider or family doctor during routine visits. This type of recruitment will take place in doctors' offices throughout the Research Triangle area. Clinicians will be informed of inclusion criteria through email and listserv announcements and be asked to mention this

clinical trial to appropriate patients and offer them a flyer/brochure with contact information. We will also recruit through UNC OB clinics and psychiatry clinics, including women's mood disorder clinics. This will be facilitated by Co-I Dr Julia Riddle, MD (Psychiatry) and study MD Dr Michael Evers, MD (OB). Finally, we will recruit participants from our Carolina Center Recruitment Registry (IRB #22-1407).

In addition to referrals through primary care providers, we will advertise the study directly to the public on websites such as (but not limited to) ClinicalTrials.gov, Research For Me, frohlichlab.org and Carolinaneurostimulation.org. We will have contact information and a summary of the clinical trial posted on the Frohlich Lab Facebook, Twitter, and other social media. Will we also be launching social media advertisement campaigns to identify potential patients. We may use radio spots, newspaper ads, billboards, and in-person recruitment as needed. We will also attend tabling events and reach out to local organizations to distribute recruitment materials. We will also be using the UNC Mass email and department listserv to send out an email that has the link to the survey.

We will send unencrypted emails to facilitate the initial contact to potential participants. Medical information is never requested per email. All medical information is recorded through HIPAA conforming Zoom meetings and surveys. We will contact potential participants up to three times by email, text message and/or phone call following completion of the prescreening survey.

Our retention strategy includes a payment schedule of up to five times per participant for a total of \$600 if all study sessions are attended. The participant will receive payment in the form of a Digital Reward via the Tango system, a platform available through UNC-CH institutional access designed for the compensation of research subjects. The Tango system gives participants access to a large catalog of gift cards, including MasterCard gift cards, from which they can choose to redeem digital rewards. The compensation schedule is as follows:

- Completion of Baseline visit: \$100
- Completion of D1 visit: \$200
- Completion of D5 visit: \$200
- Completion of FUP1: \$50
- Completion of FUP2: \$50

Total possible compensation: \$600

Patients who attend screening but fail inclusion/exclusion criteria will be reimbursed \$20 for their time.

Research personnel will be easily available for the participants to contact via email or phone. The inclusion criteria state that each participant must be able to understand all the risks and benefits associated with this study. We will aim to have a specific research team member assigned to complete all sessions with the same participant. However, we will not require the same researcher to be present during stimulation sessions 2 through 5. The study team will work hard to form and maintain a rapport with the participant so they feel comfortable and willing to discuss what may be sensitive information.

As this study requires several days of participation in a row, we will allow participants to miss one stimulation session. If two stimulation sessions are missed, the participant will be withdrawn from participation in the intervention, but study assessments will continue to be administered for safety monitoring. Non-English-speaking individuals are excluded because the ability to accurately and completely communicate study information, answer questions about the study, and obtain consent in the English language are necessary, and due to resource constraints it is not feasible to engage an interpreter for language services.

6 STUDY INTERVENTION

6.1 STUDY INTERVENTION(S) ADMINISTRATION

6.1.1 STUDY INTERVENTION DESCRIPTION

PandA-tACS will be delivered using the neuroConn DC-STIMULATOR MC designed by neuroConn GmbH (Ilmenau, Germany). Several versions of this research device have been used widely as a standard tACS and tDCS device in the international neurostimulation field for over 10 years.

The DC-STIMULATOR MC provides up to 16 electrically independent, freely programmable, micro-processor-controlled constant current sources using independent channels. It meets the highest safety standards due to hardware- and software-based multistage monitoring of current path. By continuously monitoring electrode impedance it can accurately maintain the user-defined current amplitude while also detecting if there is insufficient contact with the skin, which will then automatically terminate stimulation to ensure subject safety.

The device is non-invasive. The device is not implanted and has not been designed for, and is not being used to, support or sustain human life. This device does not have a potential for serious risk to the health, safety, or welfare of the participant. There has never been an instance of serious side-effect reported due to use of transcranial brain stimulation. Previous studies in the Frohlich Lab that used this device and comparable devices have always been classified as “non-significant risk” by the UNC IRB.

The DC-STIMULATOR MC strictly adheres to a number of safety standards, including current limitation, continuous impedance monitoring to ensure good skin contact and a safe impedance level, gradual ramp up and ramp down of current to minimize discomfort and phosphenes, and polarity reversal protection. Further, it is compliant with a number of international standards including:

- CE Mark
- EN60601-1: Medical electrical equipment – part 1: General requirements for basic safety and essential performance
- EN60601-1-2: Medical electrical equipment – Part 1-2: General requirements for basic safety and essential performance – Collateral standard: Electromagnetic disturbances – Requirements and tests
- EN60601-1-6: Medical electrical requirement Part1-6: General requirements for basic safety and essential performance – Collateral standard: Usability
- EN62304: Medical device software – Software life-cycle processes
- EN62366-1: Medical devices – part 1: Application of usability engineering to medical devices
- EN ISO 10993-1: Biological evaluation of medical devices – part 1: Evaluation and testing within a risk management system process
- EN ISO 14971: Medical devices – Application of risk management to medical devices

DC-STIMULATOR MC Specifications

- currents and wave forms of up to $\pm 4,000 \mu\text{A}$
- AC current adjustable up to $8,000 \mu\text{A}$ (peak-to-peak)
- frequencies of up to $1,000 \text{ Hz}$ selectable and phase freely adjustable
- freely selectable application duration • 16-bit D/A conversion
- time resolution $< 1 \text{ ms}$ (sample rate $16,000 \text{ sps}$)
- recording of stimulation sequences with $8,000 \text{ measurements/second}$
- max. 1 % relative direct current fault tolerance

- max. 0.02 % direct current fluctuation
- current variance during stimulation < 0.02 %
- voltage limit of 30 V
- power supply via external medical power supply unit • dimensions: 420 mm x 395 mm x 170 mm (W x D x H)
- weight: 4.2 kg

DC-STIMULATOR MC Features:

- 4/8/12/16 programmable, micro-processor-controlled constant current sources using independent channels (16 channels*)
- for transcranial direct current stimulation (tDCS), transcranial alternating current stimulation (tACS), cranial electrical stimulation (CES), galvanic vestibular stimulation (GVS) and transcranial random noise stimulation (tRNS)
- 4/8 channels, capable of alternating current, bipolar stimulation possible (16 channels*)
- medical panel pc for the use and programming of stimulation modes and stimulation sequences
- various types of stimulation can be selected and combined, continuous stimulation, cyclical switching on and off of stimulation, sinusoidal stimulation (up to 1,000 Hz)
- import of any stimulation sequences into the software to control the DC-STIMULATOR MC with customer-specific signal sequences*
- high safety standard through multistage monitoring of the current path
- external trigger input* (* optional)

The DC Stimulator MC allows programming with customizable waveforms, as per the Panda-tACS personalization method.

Additionally, the neuroConn LOOP-IT device may be used as a backup intervention device. This device is the newest version of the DC Stimulator MC, featuring multiple stimulation channels as well upgraded 'closed-loop' technology allowing customizable stimulation waveforms. The LOOP-IT has very similar device safety specifications as the DC Stimulator MC:

- Number of channels: 2 pairs (2 anode, 2 cathode)
- Stimulation modes: TIF or tDCS, tACS, tRNS
- Stimulation current maximum: 8 mA
- Bandwidth: 5 kHz

6.1.2 DOSING AND ADMINISTRATION

An eyes open and eyes closed resting state HD-EEG will be performed at Baseline, which will be used to inform each participant's personalized stimulation waveform. HD-EEG will be recorded for 6 minutes (eyes open). During the eyes open EEG, participants will watch a nature movie such as 'Reefscapes'. After the participant has completed their visit, the HD-EEG data will be processed to generate the personalized waveform. Here, HD-EEG data will be transformed from time to frequency space using a Fast Fourier Transform function, and we will manually flatten the slope of the aperiodic component using signal processing methods to reflect greater excitation (a 'flatter' exponent), with the goal of restoring excitation/inhibition balance. The waveform will then be inversely transformed back into time domain to generate a 'corrected' individualized, naturalistic stimulation waveform. This individualized waveform will then be loaded into the tACS device and applied as tACS treatment with the goal to closely mimic, and restore, brain activity.

Participants will return to the Centre on stimulation Days 1-5 (Mon-Fri). Each day participants will receive 40-50 minutes of tACS treatment with their individualized waveform. The research team will first measure each participant's head using the 10-20 system to determine the electrode locations. Participants will then be fitted with the 3 electrodes for stimulation: two 5x5cm electrodes placed over F3 (left frontal cortex, standard 10-20 EEG coordinated) and F4 (right frontal cortex), and one 5x7cm electrode placed over Cz (midline, central). Electrodes will be carbon rubber, with Ten20 conductive paste applied. The HD-EEG net will be placed on top of the stimulation electrodes, to allow for recording throughout the stimulation period. During stimulation, participants will stay in a relaxed, yet experimentally controlled state, by watching a nature movie such as "Reefscapes" during stimulation. On each stimulation day, participants will receive stimulation in four blocks of ten minutes, with intermittent EEG recording throughout the session (2 minute blocks in between stimulation blocks, with EEG recording before first block and after last block), totalling 40 minutes of stimulation. Stimulation includes a ramp-up of 20 seconds and ramp down of 3 seconds. Participants will have a full-length HD-EEG (3 minutes eyes open, 2 minutes eyes closed) on D1 and on D5 of stimulation to measure brain connectivity changes following treatment.

Research personnel will be thoroughly trained and have training documented on the transcranial stimulation device and will be present during all stimulation sessions. To monitor sensations during stimulation, a stimulation sensation questionnaire will be administered after each stimulation session. Spontaneous adverse event disclosures from participants will be captured throughout the study. Additionally, adverse events that the participant may have experienced will be explicitly queried at every study visit (see 'AE Check'). For the antenatal depression group, blood pressure and pregnancy monitoring questions are asked every day of the intervention.

6.2 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING.

This study is an open-label pilot study. Therefore, there are no randomization procedures and no blinding procedures.

6.3 STUDY INTERVENTION COMPLIANCE

Full compliance with the intervention is defined as completing all five daily stimulation sessions for up to 50 minutes each day. An acceptable compliance for the purposes of study outcome is defined as completing four of five stimulation sessions. As the intervention is applied and monitored by research personnel, compliance can be directly observed.

6.4 CONCOMITANT THERAPY

Eligible participants will be permitted to receive concomitant therapy, such as psychotherapy, antidepressants, or other medications. However, eligibility for this trial includes a stable antidepressant medication dose for the past 6 weeks before the screening visits. Moreover, a new antidepressant or a new course of psychotherapy must not be started during the last 6 weeks before the screening visit. Participants who are not taking medication for the treatment of antenatal depression are also included.

To ensure that concomitant therapies are logged appropriately, participants will be requested to report any changes during the course of the study to the researchers. Furthermore, concomitant therapies (pharmacotherapy, psychotherapy) will be logged at Screening, Baseline, treatment phase (D1-D5), FUP1 and FUP2. 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), treatment indication, and treatment start and stop dates, as applicable.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTION

Discontinuation from the week of stimulation does not mean discontinuation from the study, and remaining study procedures should be completed as indicated by the study protocol. If a clinically significant finding is identified (including, but not limited to, changes from baseline) after enrollment, the investigator or qualified designee will determine if any change in participant management is needed. The study intervention (i.e., the 5 consecutive days of stimulation) will be discontinued for the following reasons:

- A participant develops significantly increased suicidal risk, as determined by an acute assessment by Dr. Riddle, Dr. Feldman, or another trained clinician.
- A participant develops significantly increased mania, as determined by an acute assessment by Dr. Riddle, Dr. Feldman, or another trained clinician.
- The participant misses more than a single day of stimulation
- 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 as determined by Dr. Riddle, Dr. Feldman, or another trained clinician.
- The participant meets any exclusion criteria (either newly developed or not previously recognized).

In every instance where a participant does not complete the study, the Investigator will document the primary reason for discontinuation in the subject's records.

For subjects that are discontinued by the Investigator, the Investigator must notify them of their discontinuation from the study in writing.

All participants are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice. However, if a participant expresses a desire to withdraw their consent for the study, the site should attempt to obtain written documentation for their study records.

If the participant withdraws from the intervention, the participant will be contacted and invited for FUP. These assessments are not intended as a clinical follow-up with the participants but serve research purposes only.

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

The reason for participant discontinuation or withdrawal from the study will be recorded on the Study Discontinuation Case Report Form (CRF). Subjects who sign the informed consent form and are randomized but do not receive the study intervention may be replaced. Subjects who sign the informed consent form, and are randomized and receive the study intervention, and subsequently withdraw, or are withdrawn or discontinued from the study, will not be replaced.

7.3 LOST TO FOLLOW-UP

Participants who miss a study visit, are unable to be contacted by the study site staff, and do not return to a subsequent visit within the allocated timeframe will be considered lost to follow-up.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit within the allowed visit window and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and/or e-mails, and if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts will be documented in the participant's study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up. The reason for participant withdrawal from the study will be recorded on the Study Discontinuation Case Report Form (CRF).

Missing data will be managed in a way appropriate to each statistical test. See statistical analysis section for details.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 ELIGIBILITY ASSESSMENTS

Medical chart review will be conducted following an eligible pre-screen determination (before screening) to review pregnancy-related inclusion/exclusion criteria.

The following clinical evaluations are administered at the Screening (virtual) visit for all participants. The assessments must be completed within 30 days of enrollment to determine study eligibility:

- **Columbia Suicide Severity Rating Scale (C-SSRS)** – a standardized measure to assess suicide risk
- **Demographics Form** – A form to capture basic participant demographics
- **The Diagnostic Interview for Anxiety, Mood, and OCD and Related Neuropsychiatric Disorders (DIAMOND)** - a diagnostic interview tool used to confirm diagnosis of MDD for the antenatal depression population, as well as assessment of alcohol and substance use disorders, bipolar, psychotic disorders, and anorexia nervosa. The following DIAMOND modules will be incorporated for the antenatal depression population: Social Anxiety Disorder (Social Phobia), Panic Disorder, Agoraphobia, Generalized Anxiety Disorder, Manic/Hypomanic Episode, Persistent Depressive Disorder (Dysthymia), Major Depressive Episode, Obsessive Compulsive Disorder, Potentially Traumatic Event, Post-Traumatic Stress Disorder, Anorexia Nervosa, Substance Use Disorder, Delusions, Hallucinations. We will use the DIAMOND full form for antenatal depression population. The full DIAMOND Self-Report Screener will be given in prescreen for healthy controls only. This is a shortened version of the DIAMOND which uses 30 questions to summarize domains of the DIAMOND, scored as 'yes/no'. If a participant answers 'yes' to any module, the corresponding full DIAMOND module will be administered to confirm. For the antenatal depression population, only the exclusionary modules of the DIAMOND self report screener will be included.

For the antenatal depression population only:

- **Hamilton Depression Rating Scale (HDRS-17)** - a structured clinical interview that is commonly used to determine the severity of depression symptoms. The HDRS-17 has proven reliability and validity and is the most widely used scale in clinical trials of depression. Scores on the HDRS-17 are clinically meaningful, with established clinical cut-offs.

- **Maudsley Staging Method (MSM)** - a multidimensional model that helps define and stage treatment resistance in unipolar depression on a scale of 3 to 15 (mild = 3-6; moderate = 7-10; severe=11-15).
- **Birth Outcomes Chart Review form**– A form to capture basic clinical history including pregnancy complications

8.2 EFFICACY ASSESSMENTS

The following evaluations are administered after enrollment at Baseline and D5 for all participants to support the determination of efficacy:

- The full **HD-EEG** will be completed at Baseline, D1, and D5 to provide an in-depth measure of brain activity and connectivity, allowing measurement of changes following the intervention. During HD-EEG, we will measure the participant's head and a cap with 128 EEG measurement electrodes will be placed on the participant's head. At Baseline, EEG will be recorded for 6 minutes eyes open (to create the personalized waveform). At D1 and D5, EEG will be recorded for 3 minutes during resting eyes open, where participants will stare at a fixation cross, and for 2 minutes during eyes closed, where participants will rest with their eyes closed. HD-EEG will also be collected throughout the stimulation period in two minute blocks in between stimulation blocks by placing the cap over the stimulation electrodes. This will provide preliminary information about how brain activity changes over the course of the intervention.

The following additional evaluations are administered at D1, D5, FUP1 and FUP2 for the antenatal depression population:

- **The Hamilton Depression Rating Scale (HDRS-17)** is a standard instrument to measure change in symptom severity in people with MDD (Hamilton, 1960). A score of 0-7 is accepted as within the normal range, 8-13 indicates mild depression, 14-18 is considered moderate depression, and a score of 19 or higher indicates severe depression (Ehde, 2018). The minimum and maximum scores on the HDRS-17 are 0 and 52, respectively. A HDRS-17 inclusion cutoff of >8 was chosen for this study as this is the cutoff for mild depression. MDD response rate is defined as $\geq 50\%$ reduction rate in HDRS-17 score and MDD remission rate is defined as $\text{HDRS-17} \leq 7$.
- **Edinburgh Postnatal Depression Scale (EPDS)** - this is a self-administered questionnaire designed to screen for potential symptoms of depression during pregnancy and the postpartum period. This scale consists of 10 short statements, where the patient checks off one of four possible answers about how they have been feeling for the past week. Responses are scored from 0-3, where total scores above 12 or 13 are likely to be suffering from depression (Cox et al., 1987).
- **Snaith-Hamilton Pleasure Scale (SHAPS)** – The SHAPS (Snaith et al., 1995) is a 14-item scale which assesses for anhedonia (the inability to experience pleasure) where each item has a set of four response categories (definitely agree=1, agree=2, disagree=3, definitely disagree=4). Participants are considered to be experiencing mild anhedonia if they disagree with three or more statements. Participants can agree or disagree on a minimum of 0 and maximum of 14 statements.

- **Clinical Global Impression Scale (CGI)** – measure of symptom severity, treatment response and the efficacy of treatments. The CGI involves a single question for the clinician, who rates how mentally ill the patient is, on a scale from 1 (normal), 2 (borderline mentally ill), 3 (mildly ill), 4 (moderately ill), 5 (markedly ill), 6 (severely ill) and 7 (among the most extremely ill patients) (Busner & Targum, 2007).
- **Quality of Life Enjoyment and Satisfaction Questionnaire (Q-LES-Q-SF)** is a self-report measure of enjoyment and satisfaction in daily functioning (Endicott et al., 1993). The total score ranges from 14 to 70, and is expressed as a percentage of the maximum total score that is achievable, where a percentage score ranging from 70-100 is generally indicative of a normal population (Lee et al., 2014).

8.3 SAFETY AND OTHER ASSESSMENTS

The following assessments are conducted for all participants:

- The **Columbia-Suicide Severity Rating Scale (C-SSRS) (Screening)** is administered at Screening (virtual) to assess suicidal ideation and determine study eligibility. It is a standardized method to assess suicide risk severity. Only participants deemed at low suicide risk (defined for this study as no active suicidal ideation in the past month and no suicide attempts, preparatory actions, or significant non-suicidal self-harm in the previous 2 years) will be enrolled. The **C-SSRS (Since Last Visit)** is administered during treatment (D1, D3, D5), FUP1 and FUP2 to assess suicidal ideation and provide safety oversight.

In addition, the answers to suicide-related questions on the HRDS-17 will be monitored. Based on these sources of information, clinical personnel will decide if an acute assessment is required. Acute assessment will be performed by a trained clinician and 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, the clinician will assist in establishing a care plan. If needed, an expedited referral to UNC Psychiatry can be coordinated. If at any point during the assessment, the participant is deemed an imminent risk of harm to self or others, study personnel may enlist the aid of first responders to ensure that the participant is safely escorted to the Emergency Department for further care. Dr. Riddle, Dr Feldman, or another trained clinician will decide if participation should be stopped after the acute assessment. If the participant is hospitalized, their participation will end.

- To assess mania, participants will be asked three questions from the DIAMOND Manic/Hypomanic module at screening, D1, D3, D5, FUP1 and FUP2 (**‘Mania Assessment’**). The Mania Assessment will be used in combination with the DIAMOND to assess for mania and therefore bipolar disorders. After administration at the initial screening, the questions will be phrased as ‘since last study visit/follow-up session’. If participants answer yes to any of these questions, Dr Riddle, Dr Feldman, or a trained clinician will follow up with the participant using the Young Mania Rating Scale to support determination of whether they have developed mania. If any participants are found by the clinician to have developed hypomania or mania, their participation will end.

- **Stimulation Sensation Questionnaire** is conducted following each stimulation session to assess potential the stimulation side effects (tingling/itching skin, warming skin, pressure on head, flickering lights; all rated absent, low, medium, or high).
- **AE Check** will be used to evaluate any adverse events (see protocol Section 8.4.1. Safety Event Reporting Timelines for details). The study personnel will also monitor and document medical and treatment changes reported by study participants from the time informed consent is obtained until the end of study participation.
- **Blood pressure monitoring** – we will measure blood pressure at baseline and before each stimulation session to ensure that participants do not have hypertension. If participants have high blood pressure prior to stimulation (i.e. D1-D5; defined for antenatal depression participants as $\geq 140/90$ mm Hg and for healthy controls as $\geq 150/90$ mm Hg; either diastolic or systolic), then a second measurement will be taken five minutes later. If the high blood pressure resolves on the second measurement, we will continue the session. If the high blood pressure does not resolve on the second measurement, we will not begin the stimulation session, and the study team will advise the participant to consult with their primary care provider (healthy controls) and/or obstetrical provider (antenatal depression group). If a participant has high blood pressure on the first measurement, which resolves on the second measurement, then the session will be continued. In the antenatal depression group, in the case that a participant is referred to their obstetrical provider with high blood pressure and are determined to not have pre-eclampsia, the intervention will be resumed where possible.

We will also measure blood pressure after each stimulation session for all participants. Elevations in blood pressure by more than 10 points systolic or 5 points diastolic that rise above the threshold defined parameters defined previously will be considered an AE.

- **Likert scales** – we will measure participant responses on three separate one item Likert scales daily prior to stimulation and at follow-up to assess anxiety, sleep quality, and fatigue. These questions will provide further insight into potential inter-individual variability which may affect response to stimulation.
 - **Anxiety Likert:** Five evenly spaced numbers each anchored to a level of anxiety (1 = not at all anxious, 2 = a little anxious, 3 = moderately anxious, 4 = very anxious, 5 = extremely anxious). Participants are given written instructions to circle the number that shows how anxious they feel at the moment. This question has been validated against the State Trait Anxiety Inventory (STAI), with a correlation of 0.75 (95% CI 0.70–0.79) (Davey et al., 2007)
 - **Sleep quality Likert:** An eleven-point numeric rating scale ranging from 0 ("best possible sleep") to 10 ("worst possible sleep"), which is significantly correlated with the Medical Outcomes Survey (MOS) Sleep Scale (sleep disturbance: $r=0.42$; quantity of sleep: $r=-0.34$; sleep adequacy: $r=-0.32$; all $p<0.001$). (Cappelleri et al., 2009)
 - **Fatigue Likert:** An eleven-point scale which asks participants: How would you rate your fatigue on a scale of 0 to 10 with 0 being 'no fatigue' and 10 being the 'worst possible fatigue?'. This scale has good test-retest reliability ($r=0.88$) and is highly correlated with the Fatigue Symptom Inventory (FSI) severity scale (0.87) and the Functional Assessment of Chronic Illness Therapy-Fatigue Scale (FACIT-F) (-0.75). (Temel et al., 2006)

In addition, the following assessments are conducted for the antenatal depression population:

- **Self-reported pregnancy monitoring/prenatal check:** fetal movement, bleeding, cramping, loss of fluid – we will ask participants daily about these self-reported pregnancy monitoring measures. If a participant notes a marked change in fetal movement, bleeding, cramping, or loss of fluid from one day to the next, we will not administer the intervention, and refer the participant to their OB.
- **Birth outcomes log** – the study team will conduct a chart review to obtain birth outcomes by 90 days following birth: including length of pregnancy in days, delivery (spontaneous, induced, cesarian (planned or acute), vaginal birth, forceps and/or vacuum extraction), gestational age at birth, Apgar 5 min, malformation, child weight at birth in grams, birth size (small for gestational age, appropriate for gestational age, large for gestational age), neonatal need for intensive care, stillbirth, maternal length of stay in hospital, maternal need for intensive care, pre-eclampsia, as well as EPDS score between 2-4 weeks post-delivery. These events will be used to support the safety of PandA-tACS in pregnancy.

8.4 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.4.1 DEFINITION OF ADVERSE EVENTS (AE)

21 CFR 812 regulations that govern clinical trials of medical devices do not include a definition of an adverse device effect and adverse event. Instead, ISO 14155 definitions of adverse device effects (ADE) and adverse events (AE) are used in this protocol.

- AE: any untoward medical occurrence, unintended disease or injury, or untoward clinical signs (including abnormal laboratory findings) in subjects, users or other persons, whether or not related to the investigational medical device; includes events related to investigational medical device or the comparator and events related to the procedure involved.
- ADE: adverse event related to the use of an investigational medical device, including adverse events resulting from insufficient or inadequate instructions for use, deployment, implantation, installation, or operation, or any malfunction of the investigational medical device, or any event resulting from use error or from intentional misuse of the investigational medical device.

8.4.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

21 CFR 812 regulations that govern clinical trials of medical devices limit their guidance to collection and reporting requirements of unanticipated adverse device effects (UADEs). Therefore, ISO 14155 definition of a serious adverse event (SAE) is used in this protocol.

- SAE is an adverse event that:
 - led to death,
 - led to serious deterioration in the health of the subject, that either resulted in:
 - a life-threatening illness or injury, or
 - a permanent impairment of a body structure or a body function, or
 - in-patient or prolonged hospitalization, or
 - medical or surgical intervention to prevent life-threatening illness or injury or permanent impairment to a body structure or a body function,
 - led to foetal distress, foetal death or a congenital abnormality or birth defect

- Note: Planned hospitalization for a pre-existing condition, or a procedure required by the CIP, without serious deterioration in health, is not considered a serious adverse event.
- UADE is any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

8.4.3 CLASSIFICATION OF AN ADVERSE EVENT

8.4.3.1 SEVERITY OF EVENT

Safety events are assessed by an investigator using the following grading system:

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

8.4.3.2 RELATIONSHIP TO STUDY INTERVENTION

All safety events must have their relationship to study intervention assessed by the investigator who examines and evaluates the participant based on temporal relationship and his/her 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 intervention). However, other factors may have contributed to the event (e.g., the participant’s clinical condition, other concomitant events). Although an event may rate only as “possibly related” soon after discovery, it can be flagged as requiring more information and later be upgraded to “probably related” or “definitely related”, as appropriate.

- **Unlikely to be related** – A clinical event, including an abnormal laboratory test result, whose temporal relationship to study intervention administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the study intervention) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).
- **Not Related** – The event 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.

8.4.3.3 EXPECTEDNESS

The study investigator(s) are responsible for determining whether an AE/ADE is expected or unexpected. An AE/ADE 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, or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

8.4.4 SAFETY EVENT REPORTING

8.4.4.1 SAFETY EVENT REPORTING TIMELINES

The occurrence of AE, ADE, SAE, or UADE may come to the attention of study personnel during the study visit, or the study participant may report it outside of the scheduled study visit. Study team records all safety events that occur any time after informed consent is obtained until 7 days (for AEs and ADEs) or 30 days (for SAEs and UADEs) after the last day of study participation.

All safety events are captured on the appropriate case report form (CRF). Information to be collected includes event description, date of onset, investigator assessment of seriousness, severity, and expectedness, relationship to study intervention (assessed only by investigators with the training and authority to make a diagnosis), date of resolution/stabilization of the event, and associated treatment.

SAEs must be reported on the appropriate CRF within 24hrs of becoming aware of the event.

Any medical condition that is present at the time that the participant is screened is considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it is recorded as an AE or ADE.

Changes in the severity of AEs and ADEs are documented to allow an assessment of the duration of the event at each level of severity. ADEs and AEs characterized as intermittent require documentation of onset and duration of each episode.

8.4.5 UNANTICIPATED ADVERSE DEVICE EFFECT REPORTING

The study investigator shall complete an Unanticipated Adverse Device Effect (UADE) Form and submit to the reviewing Institutional Review Board (IRB) as soon as possible, but in no event later than 7 working days after the investigator first learns of the effect (OHRE SOP 1401).

REPORTING EVENTS TO PARTICIPANTS

Any new information gained during the study that may affect a participant's willingness to continue in the study will be reported to all currently enrolled participants via individual phone calls, during in-clinic visits, or aggregate written communication, as appropriate.

8.4.6 REPORTING OF PREGNANCY (FOR HEALTHY CONTROL PARTICIPANTS)

In relation to healthy control participants only: pregnancy is not an adverse event. Healthy controls of childbearing potential are asked if they are pregnant or plan to become pregnant before the stimulation session. Furthermore, they are asked to use an appropriate method of birth control for the duration of trial participation. All participants of childbearing potential will be asked to undertake a pregnancy test at baseline. Importantly, there is no evidence that suggests tACS would interfere with pregnancy (Konstantinou et al., 2020). If a healthy control participant becomes pregnant during the treatment period (according to self-report), the study treatment is discontinued and the participant is asked to remain in the study and followed for safety follow-up until the pregnancy outcome. If the participant becomes pregnant after completion of the 5 day study treatment, the participant should complete the remaining study visit, and will be followed until pregnancy outcome.

8.5 UNANTICIPATED PROBLEMS

8.5.1 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; **and**
- Is at least possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); **and**
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

This definition could include an unanticipated adverse device effect, any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects (21 CFR 812.3(s)).

8.5.2 UNANTICIPATED PROBLEM REPORTING

The investigator will report unanticipated problems (UPs) to the reviewing Institutional Review Board (IRB). The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;

- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- UPs that are serious adverse events (SAEs) will be reported to the reviewing IRB within 7 calendar days of becoming aware of the event.
- Any other UP will be reported to the IRB within 7 calendar days of the investigator becoming aware of the problem.

8.5.3 REPORTING UNANTICIPATED PROBLEMS TO PARTICIPANTS

Any new information gained during the study that may affect a participant's willingness to continue in the study will be reported to all currently enrolled participants via individual phone calls, during in-clinic visits, or aggregate written communication, as appropriate.

9 STATISTICAL CONSIDERATIONS

9.1 STATISTICAL HYPOTHESES

We do not have statistical hypotheses for endpoints, as this study is only a pilot study testing safety, feasibility, and tolerability in a small sample. We will report descriptive statistics for each endpoint as appropriate.

9.2 SAMPLE SIZE DETERMINATION

This study is a pilot study with safety, feasibility, and tolerability as primary outcomes. Therefore, it will not be powered for efficacy determination. The sample size of n=5 healthy controls (Phase I) and n=5 people with antenatal depression (Phase II) was chosen based on the hypothesized feasible recruitment rate over a 12 month period (less than one participant per month). The safety, feasibility, tolerability, and preliminary efficacy results of this trial will inform a larger scale trial designed to measure efficacy.

9.3 POPULATIONS FOR ANALYSES

Every effort will be made to ensure all enrolled participants complete all study sessions as described in this protocol. As previously stated in Section 7 Study Intervention Discontinuation and Participant Discontinuation/Withdrawal, enrolled participants who do not receive any study intervention sessions may be replaced. If a participant completes less than four stimulation sessions, they will not be replaced, given the focus on protocol feasibility. With this population for our analysis plan, we anticipate having data from five healthy controls and five people with antenatal depression that are eligible for analysis.

9.4 STATISTICAL ANALYSES

9.4.1 GENERAL APPROACH

Continuous data will be described using means and standard deviations, while categorical data will be described using counts/percentages. There will be no pre-specified covariates described in this protocol. Data will be assessed for normality and, if deemed necessary, corrective procedures will be applied (e.g., log normalization).

9.4.2 ANALYSIS OF THE PRIMARY ENDPOINT(S)

We do not have statistical analyses for endpoints, as this study is only a pilot study testing safety, feasibility, and tolerability in a small sample. We will report descriptive statistics for each endpoint as appropriate, in each of Phase I and Phase II.

9.4.3 ANALYSIS OF THE SECONDARY ENDPOINT(S)

N/A

9.4.4 SAFETY ANALYSES

As discussed in **Section 8.3**, Safety and Other Assessments safety will be assessed with the C-SSRS, an adverse effects questionnaire, and mania questions from the DIAMOND Manic/Hypomanic episode module, followed by the YMRS.

The C-SSRS will be used to assess suicide risk. If a participant's responses indicate an elevated risk, the participant will be contacted, and an acute assessment will be completed if deemed necessary by Dr. Riddle, Dr Feldman, or another trained clinician (as described above). Any verified increases in suicidal ideation will be described in counts/percentages. The C-SSRS is administered at the screening visit, D1, D3, D5, FUP1 and FUP2. After D1, the C-SSRS will be phrased as 'since last visit'.

The stimulation sensation questionnaire will be administered at D1-D5 after stimulation. This questionnaire asks participants to rate the following sensations possibly related to tACS as absent, low, medium, or high: "tingling/itching skin", "warming skin", "pressure on head", "flickering lights". Intensity of sensory experiences and adverse effects will be described with mean and standard deviation.

To assess mania, participants will be asked three questions from the DIAMOND Manic/Hypomanic module at screening, D1, D3, D5, FUP1 and FUP2 (Mania Assessment). After D1, the mania check will be phrased as 'since last visit'. If participants answer yes to any of these questions, Dr Riddle, Dr Feldman, or a trained clinician will follow up with the participant with the Young Mania Rating Scale (YMRS) to determine whether they have developed mania.

The AE Check will be administered by research personnel at every stimulation visit and follow-up and whenever a participant spontaneously reports a potential AE during trial participation. This check involves the following questions: "*Have you had any adverse events since our last visit? Have you been in the hospital? Seen a doctor, or otherwise sought medical care?*".

For the antenatal depression population, we will obtain medical history after pre-screening (before screening) and birth outcomes following the study using chart review. We will only enroll participants who have been seen through UNC to ensure chart availability. We will follow-up at 2 weeks post intervention, at 4-6 weeks post-delivery, and then conduct chart review within 90 days of birth. For birth outcomes, we will obtain length of pregnancy in days, delivery (spontaneous, induced, cesarian (planned or acute), vaginal

birth, forceps and/or vacuum extraction), gestational age at birth, Apgar 5 min, malformation, child weight at birth in grams, birth size (small for gestational age, appropriate for gestational age, large for gestational age), neonatal need for intensive care, stillbirth, maternal length of stay in hospital, maternal need for intensive care, pre-eclampsia, as well as EPDS score between 2-4 weeks post-delivery. These metrics will be reviewed in the context of general pregnancy outcomes.

9.4.5 BASELINE DESCRIPTIVE STATISTICS

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

9.4.6 PLANNED INTERIM ANALYSES

This intervention is organized into two phases (Phase I: healthy controls and Phase II: antenatal depression). We will undertake an interim analysis for safety, feasibility, and tolerability outcomes once Phase I of the data is complete. Outcomes of this interim analysis will inform continuation onto Phase II of the intervention in antenatal depression participants. The study Investigators will review the outcomes of Phase I with the medical monitor before moving to Phase II.

9.4.7 TABULATION OF INDIVIDUAL PARTICIPANT DATA

If individual participant data is listed, it will be de-identified and used only for the purpose of describing the sample in terms of clinical characteristics.

9.4.8 EXPLORATORY ANALYSES

We will report descriptive statistics for each endpoint as appropriate, in each of Phase I and Phase II.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

10.1.1 INFORMED CONSENT PROCESS

10.1.1.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

Informed consent is a process that is initiated prior to the individual agreeing to participate in the study and continues throughout the individual's study participation. Extensive discussion of risks and possible benefits of tACS will be provided to the participants and their families. We apply for a waiver for written consent for our pre-screening survey and our screening visit. For the pre-screening survey, interested potential participants will indicate their consent to answering the questions as part of the survey. For the screening visit, verbal consent will be obtained and documented at the beginning of the visit. Written consent for study participation will be obtained as explained in the following section below. All consent forms will be IRB-approved and updated with any new information as modifications are made throughout the study.

An IRB-approved consent form describing in detail the study intervention, study procedures, and risks is given to the participant and written documentation of informed consent is required prior to starting intervention/administering study intervention. The following consent materials are submitted with this protocol:

- Pre-Screening Consent (part of the Pre-Screening Questionnaire)
- Informed Consent Form

10.1.1.2 CONSENT PROCEDURES AND DOCUMENTATION

Participants interested in completing the pre-screening survey, designed to assess preliminary eligibility in the study, must provide consent to participate in the pre-screening questionnaire. The consent is given by selecting the appropriate checkbox at the beginning of the pre-screening survey. Participants that are successfully pre-screened will be invited to a virtual Screening visit, and will be sent the consent form for review in their own time prior to the virtual screen.

Verbal consent will be obtained at beginning of the virtual screening visit. Further, verbal consent will be obtained to record the screening (optional). Recording the screening will allow retrospective review and/or validation for inter-rater reliability between clinicians. Only the audio of the HIPAA-compliant zoom screening will be retained. Following screening, for participants who are deemed eligible, informed consent will be collected at the conclusion of the Screening visit as an electronic form through Veeva SiteVault. Extensive discussion of risks of transcranial current stimulation will take place between an investigator and potential participant before the informed consent form (eICF) is signed. The eICF describes, in detail, the study intervention, device, procedures, and risks and is shared with a potential participant for review. The researcher and patient will review the study in its entirety by reviewing the eICF together. At several intervals during the consent review the researcher will ask the participant questions that assess the comprehension of the information in the eICF. If the participant is unsure or does not understand the eICF content, the researcher will return to that section and more carefully explain the information. Participants must electronically sign the eICF prior to any procedures taking place. Participants will have time to think about or discuss the study with their surrogates prior to agreeing to participate. Participants may withdraw consent at any time throughout the course of the trial. A copy of the eICF will be given to the participant for their records.

The researcher will document in source that the potential participant had sufficient time to ask questions about study participation, what the questions and answers were, that participant received a signed copy of the eICF, version of the eICF signed by the patient and researcher, and date and time of the informed consent. All eICFs will be IRB-approved and will be updated with new information as modifications are made throughout the study.

10.1.1.2 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 study participants and the Institutional Review Board (IRB) and funding body and will provide a written notification, documenting 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, and unacceptable risk to participants
- Insufficient compliance to protocol requirements

- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the IRB and/or Food and Drug Administration (FDA).

10.1.3 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating Investigators and their staff. This confidentiality is extended to cover the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the PI.

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

Authorized representatives of the representatives of the Institutional Review Board (IRB), regulatory agencies, or medical device manufacturer supplying study product may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at the clinical site for internal use during the study. 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 reviewing IRB, Institutional policies, or funding requirements.

Study participant research data, which is used for purposes of statistical analysis and scientific reporting, will be stored in locked filing cabinets at the study site. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical sites and by research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived.

Certificate of Confidentiality

To further protect the privacy of study participants, a Certificate of Confidentiality will be issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

10.1.4 FUTURE USE OF STORED SPECIMENS AND DATA

Data collected for this study will be stored in locked filing cabinets at the study site and only authorized staff will have access to the data.

No biological specimen or genetic data will be collected as part of this study.

10.1.5 KEY ROLES AND STUDY GOVERNANCE

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

Principal Investigator	Medical Monitor
<i>Dr Flavio Frohlich, PhD MSc</i> <i>ETH MA</i> Director, Carolina Center for Neurostimulation; Professor, Department of Psychiatry; Associate Vice Chair of Research	<i>Dr Michael Evers, MD</i> Assistant Professor, General Obstetrics, Gynecology, and Midwifery Clinical Assistant Professor, Radiology
<i>UNC Chapel Hill</i>	<i>UNC Chapel Hill</i>
<i>100 Market Street, Suite 1, Chapel Hill, NC 27516</i>	<i>1181 Weaver Dairy Rd #150, Chapel Hill, NC 27514</i>
<i>(919) 966-9929</i>	<i>919-843-7850</i>
<i>Flavio_frohlich@med.unc.edu</i>	<i>michael_evers@med.unc.edu</i>

10.1.6 SAFETY OVERSIGHT

A DSMB will not be established for this study. This study will be overseen by a Medical Monitor as described above.

10.1.7 CLINICAL MONITORING

Clinical site monitoring is conducted to ensure that the rights and well-being of trial participants are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with International Conference on Harmonisation Good Clinical Practice (ICH GCP), and with applicable regulatory requirement(s).

This study will be overseen by a Medical Monitor as described above. Independent audits will not be conducted by the Medical Monitor unless requested.

10.1.8 QUALITY ASSURANCE AND QUALITY CONTROL

The study Investigators will ensure appropriate training for each member of the team prior to initiation of the study at the investigational site. This training will address topics such as the protocol, subject selection criteria, AE management, post-treatment care, and follow-up, indications and contraindications for the use of the device, device handling, and device instrumentation.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database. Any missing data or data anomalies will be communicated to the site for clarification/resolution.

The study Investigators 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.

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and inspection by local and regulatory authorities.

10.1.9 DATA HANDLING AND RECORD KEEPING

10.1.9.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

Data will primarily be captured in REDCap as a source form. Any data recorded in the electronic case report form (eCRF) derived from source documents should be consistent with the data recorded on the source documents.

Clinical data (including adverse events (AEs), concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into REDCap. Clinical data will be entered directly from any paper source documents.

10.1.9.2 STUDY RECORDS RETENTION

Study documents should be retained for a minimum of 2 years after the last approval of a marketing application in an International Conference on Harmonisation (ICH) region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the study intervention. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the PI, if applicable.

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

10.1.10 PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the clinical trial protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), or Manual of Procedures (MOP) requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH GCP:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the site investigator to use continuous vigilance to identify and report deviations in a timely manner. All deviations must be addressed in study source documents. Protocol deviations must be sent to the reviewing Institutional Review Board (IRB) per their policies. The site investigator is responsible for knowing and adhering to the reviewing IRB requirements.

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, if applicable, were notified. The PI or delegated Co-I will review the information and initial once approved. A completed copy of the Protocol Deviation Form will be maintained in the regulatory file, as well as in the participant's source document. The site PI/study staff will be responsible for knowing and adhering to their IRB requirements.

10.1.11 PUBLICATION AND DATA SHARING POLICY

This trial will be registered at ClinicalTrials.gov, and results from this trial will be submitted to ClinicalTrials.gov. In addition, every reasonable 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, such as by the medical device industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The University of North Carolina IRB has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

10.2 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
ANCOVA	Analysis of Covariance
CFR	Code of Federal Regulations
CGI	Clinical Global Impression Scale
CLIA	Clinical Laboratory Improvement Amendments
CMP	Clinical Monitoring Plan
COC	Certificate of Confidentiality
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
C-SSRS	Columbia Suicide Severity Rating Scale
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DIAMOND	Diagnostic Interview for Anxiety, Mood, and OCD and Related Neuropsychiatric Disorders
DSMB	Data Safety Monitoring Board
DRE	Disease-Related Event
Dx	Diagnosis
EC	Ethics Committee
eCRF	Electronic Case Report Forms

ECT	Electroconvulsive therapy
EEG	Electroencephalography
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
FFR	Federal Financial Report
FUP	Follow-up
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
GWAS	Genome-Wide Association Studies
HD-EEG	High-density electroencephalography
HDRS-17	Hamilton Depression Rating Scale
HIPAA	Health Insurance Portability and Accountability Act
Hz	Hertz
IB	Investigator's Brochure
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IDE	Investigational Device Exemption
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISM	Independent Safety Monitor
ISO	International Organization for Standardization
ITT	Intention-To-Treat
LSMEANS	Least-squares Means
MDD	Major depressive disorder
MedDRA	Medical Dictionary for Regulatory Activities
MHx	Medical history
MOP	Manual of Procedures
MSDS	Material Safety Data Sheet
MSM	Maudsley Staging Method
NCT	National Clinical Trial
NIH	National Institutes of Health
NIH IC	NIH Institute or Center
OHRP	Office for Human Research Protections
PandA-tACS	Personalized antenatal depression aperiodic transcranial alternating current stimulation
PI	Principal Investigator
QA	Quality Assurance
QC	Quality Control
Q-LES-Q-SF	Quality of Life Enjoyment and Satisfaction Questionnaire
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SHAPS	Snaith-Hamilton Pleasure Scale
SMC	Safety Monitoring Committee
SOA	Schedule of Activities
SOC	System Organ Class
SOP	Standard Operating Procedure
tACS	Transcranial alternating current stimulation
TMS	Transcranial magnetic stimulation
UNC	University of North Carolina
UP	Unanticipated Problem
US	United States
YMRS	Young Mania Rating Scale

The table below is intended to capture changes of IRB-approved versions of the protocol, including a description of the change and rationale. A Summary of Changes table for the current amendment is located in the Protocol Title Page.

[illegible]

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12 APPENDIX

12.1 Mania Assessment questions

- *In the last month, have you had a period of time, lasting at least four days, when your mood was so good or elevated, like you were on top of the world, that it caused problems for you, or people thought you weren't your usual self?*
- *In the last month, have you had a period of time lasting at least four days, when you felt so good about yourself, or you felt so powerful or capable of taking on new projects, that it caused problems for you, or people thought you weren't your usual self?*
- *In the last month, have you had a period of time, lasting at least four days, when your mood was so irritable or cranky that it caused problems for you, or people thought you weren't your usual self?*

12.2 AE Check questions

- *Have you had any adverse events since our last visit?*
- *Have you been in the hospital?*
- *Seen a doctor, or otherwise sought medical care?*

12.3 Birth outcomes

- Length of pregnancy in days
- Delivery (spontaneous, induced, cesarian (planned or acute), vaginal birth, forceps and/or vacuum extraction)
- Gestational age at birth
- Apgar 5 min
- Malformation
- Child weight at birth in grams
- Birth size (small for gestational age, appropriate for gestational age, large for gestational age)
- Neonatal need for intensive care
- Stillbirth
- Maternal length of stay in hospital
- Maternal need for intensive care
- Pre-eclampsia
- EPDS score between 2-4 weeks post-delivery