Effects of Transcranial Alternating Current Stimulation (tACS) on Memory Recall and Sleep-EEG in Healthy Elderly Participants

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Master Protocol Document

Title	Effects of Transcranial Alternating Current Stimulation (tACS) on Memory Recall and Sleep-EEG in Healthy Elderly Participants
Sub-Title	A Double-Blind, Controlled Cross-Over Pilot Study
Principal Investigator	Flavio Frohlich, PhD, Carolina Center for Neurostimulation, Department of Psychiatry, UNC School of Medicine, Chapel Hill, NC (also serving in the role of statistical expert)
Co-Investigators	Andrea Seiler, MD, Carolina Center for Neurostimulation, Department of Psychiatry, UNC School of Medicine, Chapel Hill, NC
Research Sites	University of North Carolina at Chapel Hill
Version Number 1.4	08/15/2023

I have read, understood, and approved this version of the protocol.

Principal Investigator: _____ Date: _____

Table of Version Changes

Previous Version No.	Affected Sections	Summary of the Changes to the Protocol	Reason for Changes
1.0	**1. Synopsis** **2. Aims** **3. Study design** **4. Eligibility criteria** **5. Treatment design** **6. Schedule (Study Visits)**	**1. Changed aims (added associative memory, adapted everything according to clinicaltrials.gov), changed outcomes and statistical plan accordingly; changed exclusion (no AD8 due to already high count of questionnaires)** **2. Changed aims according to clinicaltrials.gov. Made sleep more concrete (only spindles and slow waves are outcomes); other neuropsychological outcomes than memory secondary outcomes (Dörthe thinks it is okay)** **3. Added telephone contacts explicitly; Changed Electrodes to F7/P7 and F8/P8** **4. No AD8** **5. Added Word association test** **6. Added associative memory, changed question about alcohol and psychotropic substances/drugs for follow-up visits (not last 3m, but last 24h), RVDLT only during Visit 2, and recall immediately after encoding (to win time for analysis of y-frequency)**	
1.1	**1. Measurement Design, Enrollment, Study Design** **2. Everywhere** **3. Section 7**	**1. RVDLT for y-Frequency on Visit 1 instead of Visit 2 (to have more time for analysis)** **2. Changed Phonematic/Verbal Fluency to Letter Fluency (to have the same vocabulary** **3. Changed Timepoint of Visit 5 from Day 5-9 after Stimulation to Day 6-9 after Stimulation (Visit 4 and 5 not on the same day, and Visit 4 already on Day 5 after Stimulation)**	
1.2		**1. 4 Hours of Sleep EEG is not an Exclusion Criteria (we want to find out how many manage to do it)** **2. Not Sham, but Control Frequency (21 Hz – 10 Hz worsens Auditory Attention, 20 Hz is harmonic; also: Closer to 40 Hz, therefore similar Side Effects, but not yet Gamma). Text from Modification: We are now tightening the scientific contrast such that we can draw conclusions about the effect of a specific stimulation frequency,**	

		WHEREAS OUR PREVIOUS DESIGN ONLY ALLOWED TO DRAW CONCLUSIONS ABOUT THE EFFECT OF STIMULATION IN GENERAL. THUS, WE NOW USE A CONTROL FREQUENCY OF 21HZ INSTEAD OF THE ACTIVE SHAM STIMULATION. 3. TACS AMPLITUDE IS INDIVIDUALIZED (SAME AMPLITUDE FOR BOTH CONDITIONS; HAS TO BE SUPPORTED FOR 1H, THRESHOLDING FOR 21 AND 40 HZ, LOWER AMPLITUDE IS TAKEN) 4. NO INDIVIDUALIZED GAMMA, BECAUSE NO CLEAR/SINGLE PEAK DURING RVDLT ACCORDING TO JIMIN'S ANALYSIS 5. ELECTRODE POSITIONING FOR LEAST SIDE EFFECTS: T7/8, P7/8 6. ACTIGRAPHY ADDED TO SEE IF STIMULATION CHANGES ACTIVITY PATTERN. TEXT FROM MODIFICATION: ACTIGRAPHY: WEARING AN ACTIGRAPHY WRISTBAND. PARTICIPANTS ARE ASKED TO WEAR A WRIST-WORN ACCELEROMETER FROM VISIT 1 TO VISIT 6. THE DEVICE RECORDS REST AND ACTIVITY PHASES BY MEANS OF AN ACCELEROMETER, THUS ALLOWING CONCLUSIONS TO BE DRAWN ABOUT THE SLEEP-WAKE RHYTHM. WE PURSUE AN ADDITIONAL EXPLORATORY GOAL WITH ADDING ACTIGRAPHY: SUPPLEMENTAL DATA IN ADDITION TO THE ALREADY APPROVED EEG WITH REGARDS TO THE NEUROPHYSIOLOGY OF MEMORY CONSOLIDATION SLEEP AFTER 40 HZ STIMULATION (COMPARED TO 21HZ AND COMPARED TO NIGHTS WITHOUT STIMULATION IN BETWEEN): THE DEVICE IS WORN ON THE WRIST (LIKE A WATCH), IT IS LIGHTWEIGHT, AND IT DOES NOT NEED TO BE RECHARGED DURING THE SCHEDULED WEARING PERIOD. 7. MOTOR THRESHOLDING (TEXT FROM MODIFICATION): SINCE THE ORIGINAL DESIGN OF THE STUDY, NEW EVIDENCE SUGGESTS AN INTERESTING RELATIONSHIP BETWEEN THE EFFECT OF TACS AND BRAIN EXCITABILITY MEASURED WITH SINGLE PULSE TMS. WE WILL THEREFORE MEASURE CORTICAL EXCITABILITY BY PERFORMING MOTOR THRESHOLDING USING SINGLE PULSE TMS (TRANSCRANIAL MAGNETIC STIMULATION) DURING VISIT 2. THE PROCEDURE TAKES ONLY A FEW MINUTES, AND ONLY SINGLE PULSES WILL BE APPLIED UNTIL A MOTOR RESPONSE RESULTS AT THE HAND (=5 OUT OF 10 EVOKED POTENTIALS OF FIRST DORSAL INTEROSSEUS MUSCLE =50MV PEAK-TO-PEAK). 8. (PLANNING) PREGNANCY IS REASON FOR EXCLUSION	

1.3		**ADAPTED OUTCOMES TO THE VERSION SUBMITTED TO THE IRB (=ADDING AIM 8).** **CHANGING TYPOS IN THE SCHEDULE.**	
1.4		**- EXCLUSION CRITERIA: PSQI > 5 (INSTEAD OF >6)** **- SESSIONS: 8AM-12PM, 12PM-6PM**	

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Statement of Compliance

This study will be conducted as specified in the protocol and in accordance with the *International Conference on Harmonisation Guidelines for Good Clinical Practice* (ICH E6) and the *Code of Federal Regulations on the Protection of Human Subjects* (45 CFR Part 46).

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

If required by the IRB, the master protocol document, informed consent form(s), recruitment materials, and all participant materials will be submitted to the *Scientific Review Committee* (SRC) prior to IRB review (research.unc.edu/clinical-trials/src).

The statistical analysis plans will be consistent with guidance in CONSORT Statement [1] or STROBE Statement [2], ICMJE recommendations [3], the 2016 and 2019 statements of the American Statistical Association [4,5], and recommendations in Nature [6,7].*

All personnel involved in the conduct of this study have completed human participants protection training.

* [1] www.consort-statement.org
[2] www.strobe-statement.org
[3] www.icmje.org
[4] Wasserstein RL, et al. (2016), The ASA's Statement on p-Values, ***The American Statistician***, 70:2, 129-133
[5] Wasserstein RL, et al. (2019), Moving to a World Beyond p < 0.05, ***The American Statistician***, 73:sup1, 1-19
[6] Amrhein, et al. (2019) Scientists rise up against statistical significance, ***Nature*** 567, 305-307
[7] Editorial (2019) It's time to talk about ditching statistical significance: Looking beyond a much used and abused measure would make science harder, but better. ***Nature*** 567, 283-283.

Table of Abbreviations

AE / SAE	adverse event / serious adverse event
CFR	U.S. Code of Federal Regulations (www.eCFR.gov)
CI	confidence interval
CIOMS	Council for International Organizations of Medical Sciences (cioms.ch)
CoC	certificate of confidentiality
CONSORT	Consolidated Standards of Reporting Trials (www.consort-statement.org)
CRF	case report form
CRO	contract research organization
CSCC	UNC Collaborative Studies Coordinating Center (sites.cscc.unc.edu/cscc)
CT.gov	ClinicalTrials.gov website
DCC	data coordinating center
DSMB	data and safety monitoring board
eCRF	electronic case report form
eCTD	electronic common technical document
EEG	Electroencephalography/electroencephalogram
DOH!	I need to delete this example term (and others not used in this protocol) from this table
FDA	U.S. Food and Drug Administration (www.fda.gov)
GCP	good clinical practice
HIPAA	U.S. Health Insurance Portability and Accountability Act (www.hhs.gov/hipaa)
ICF	informed consent form
ICH	International Council for Harmonization (www.ich.org)
ICMJE	International Committee of Medical Journal Editors (www.icmje.org)
IDE	investigational device exemption
IDS	UNC Investigational Drug Services (uncids.web.unc.edu)
IND	investigational new drug application
IRB	institutional review board
MAR	missing at random criterion
MCAR	missing completely at random criterion
MNAR	missing not at random criterion
MICE	multiple imputation by chained equations
MOP	manual of procedures
MPD	master protocol document
N	number of enrolled participants
NDA	new drug application
OCT	UNC Office of Clinical Trials (research.unc.edu/clinical-trials)
OHRP	Office for Human Research Protections
PHI	protected health information
PI	principal investigator
PRC	UNC Oncology Protocol Review Committee (UNClineberger.org/protocolreview)
QA	quality assurance
RCT	randomized controlled trial
REDCap	Research Electronic Data Capture system
SD	standard deviation
SE	standard error
SOP	standard operating procedures
SRC	UNC Scientific Review Committee (research.unc.edu/clinical-trials/src)
STROBE	Strengthening Reporting of Observational Studies in Epidemiology (www.strobe-statement.org)
tACS	Transcranial Alternating Current Stimulation
TraCS	N.C. Translational and Clinical Sciences Institute (tracs.unc.edu)
UNC	The University of North Carolina
UNCH	UNC Hospitals

1. Protocol Synopsis

Title	Effects of Transcranial Alternating Current Stimulation (tACS) on Memory Recall and Sleep-EEG in Healthy Elderly Participants (SUPERCHARGE)
Study Description	The purpose of this clinical trial is to investigate the feasibility and efficacy of non-invasive transcranial alternating current stimulation (tACS) at gamma frequency in enhancing memory recall and modulating sleep network dynamics measured by at-home electroencephalography (EEG) in healthy elderly people. Eligible participants will first collect sleep EEG at home for one night to acclimate to the data collection during sleep. Participants are then randomized into first undergoing either gamma-tACS or controlcontrol tACS. Stimulation is administered in the lab during a cognitive testing battery that includes memorizing items. After a night of sleep with EEG at home, participants return to the lab the following day to measure memory recall. Memory recall is tested again by telephone five days later. This sequence of encoding during stimulation in the lab, sleep EEG at home for one night, and memory recall is then repeated for the other stimulation condition about a week later. An acigraphy watch (accelerometer) is worn during the whole study period.
Specific Aims **(objectives)**	**Aim 1:** To establish acceptability of cognitive testing during tACS in healthy elderly participants. **Aim 2:** To investigate the effect of a single session of gamma-tACS (compared to control tACS) on verbal memory (a) and associative memory (b) performance during stimulation, on the day after stimulation and after five days. **Aim 3:** To establish feasibility of at-home use of a single-channel EEG device during one night (a) and acceptability during three nights (b). **Aim 4:** To investigate the effect of a single session of gamma-tACS (compared to control tACS) on the amount of sleep spindles (a) and slow wave sleep (b) occurring during the night after the intervention. **Aim 5:** To see if there is a correlation between memory recall performance and sleep EEG features (especially spindles, slow waves). **Aim 6:** To investigate the effect of a single session of gamma-tACS (compared to control tACS) on a letter fluency task (a), executive functioning (Stroop Test (b), Trail Making Test (c)) and attention (Attentional Performance, TAP (d)). **Aim 7:** To see if the motor activity pattern (measured by actigraphy) changes after stimulation/type of stimulation. **Aim 8:** To investigate if there is a relationship between the brain excitability derived from the subjective effect of tACS and the motor threshold measured by TMS.
Target Population	**Inclusion Criteria** • >50 years old **Exclusion Criteria** • Implanted device or metal in head (including cochlear implant or other hearing aid), cardiac pacemaker or any other powered medical device • Known neurological disease from history (epilepsy, sleep disorder (insomnia, sleep apnea, restless legs syndrome,

	parasomnia), stroke or transitory ischemic attack, cognitive impairment, neurodegenerative disease (for example Alzheimer's disease, Parkinson's disease or amyotrophic lateral sclerosis), immune-mediated disease of the central nervous system, chronic infectious brain disease, brain tumor, traumatic brain injury with loss of consciousness and/or intracranial bleeding, chronic pain with the need for daily analgesic use) • Positive screening for epilepsy (questionnaire) • Pathological Montreal Cognitive Assessment (MoCA <26/30 points) • Brain surgery in the past (lifetime) • Known psychiatric disorder from history (schizophrenia (lifetime), obsessive compulsive disorder (OCD; lifetime), borderline personality disorder (lifetime), anxiety disorder (lifetime), bipolar disorder (lifetime), psychosis (lifetime), eating disorder (lifetime), depression (within the last three months) • Positive screening for anxiety disorder (GAD-7 ≥10/21 points) or positive screening for depression (PHQ-9 ≥5/27 points) • Known other relevant medical condition from history (moderate to severe chronic obstructive pulmonary disease (COPD), abnormal kidney function (defined as estimated Glomerular Filtration Rate <60ml/min), current liver disease (defined as hepatitis and/or liver cirrhosis), cancer, diabetes mellitus, cardiac disease (heart failure, myocardial infarct, atrial fibrillation and revascularization – all within the last three months) • Working in night shifts or going to bed after midnight on 3 or more nights per week • Positive screening for sleep disorder (PSQI >5/21 points) • Psychotropic treatment or illegal drugs (including cannabis) within the last three months • Indication for alcohol use disorder: AUDIT score (Alcohol Use Disorders Identification Test; screening for unhealthy alcohol use) ≥7 for females and for males ≥ 65 years or ≥8 for males <65 years • Not willing to abstain alcohol at least 24 hours before each study visit • Women who want to become pregnant, are pregnant, plant to seek or are seeking fertility treatment Reason for immediate early termination of participation • Epileptiform activity in 128-channel EEG on the first day
Numbers of Enrollees	This pilot study requires n = 28 participants who complete all aspects of the protocol. Participants are recruited and enrolled until this target is reached. We anticipate screening up to 100 participants and enrolling up to 50 participants.

Intervention	This is a single-center prospective interventional control controlled cross-over pilot study. The intervention consists of the application of tACS stimulation in the gamma-frequency range during approximately one hour of cognitive testing.
Outcome Measures	**Aim 1:** Cognitive testing during tACS completed in >80% of participants. **Aim 2:** Participants will memorize 15 words ("Rey Verbal Learning Test", (a)) and 8 word-pairs (associative verbal memory, (b)) during gamma-tACS and control tACS. They will be required to recall the words after a short delay on the same day, on the day after and after 5 days ("ultra-delayed recall"). The number of correctly remembered words (a) and word-pairs (b) will be recorded. **Aim 3:** Feasibility (a) will be considered feasible if the device has been worn for at least 4 hours during the first night for >/= 80% of participants. Acceptability (b) will be considered as given if the device has been worn for at least 4 hours during each of the three nights in >/=80% of participants. **Aim 4:** Amount of sleep spindles (a) and slow wave sleep (b) during sleep recording. **Aim 5:** Exploration of correlation between memory recall performance and sleep EEG features (especially spindles, slow waves). Difference in recall scores (verum versus sham tACS) will be correlated with differences in sleep EEG features (verum versus sham tACS). **Aim 6:** For (a) the number of correct words, of perseverations and of rule breaks will be calculated. For (b) and (c) time for test completion and errors will be calculated. For (d) percentile ranks for the following subtests will be calculated: Alertness, Go/NoGo, Divided Attention, Visual Scanning. **Aim 7:** Exploration of correlation between amount of motor activity/inactivity phases (measured by actigraphy watch) and stimulation versus no stimulation as well as gamma- versus control stimulation. **Aim 8:** Exploration of correlation between the TMS motor threshold and the maximal tolerated amplitude (according to the subjective symptoms) for tACS.
Statistical Analysis Plans for Each Aim	**Aim 1:** Proportion (percentage and 95% confidence interval) of participants with completed cognitive testing during stimulation study session. **Aim 2:** Comparison of memory and associative testing after stimulation and sham on individual and group level using ANCOVA. **Aim 3:** Proportion (percentage, 95% confidence interval) of participants with at least four hours of EEG-data in the first night (a).Proportion (percentage,

	95% confidence interval) of participants who successfully collected at least four hours of EEG for all three study nights (b). **Aim 4:** Comparison of sleep spindles (a) and slow wave sleep (b) derived from sleep EEG for verum versus control tACS using ANCOVA. **Aim 5:** Exploratory investigation of correlations (Pearson's) between stimulation induced changes in memory recall performance and sleep EEG features. **Aim 6:** Comparison of test results after stimulation and sham on individual and group level using ANCOVA. **Aim 7:** Exploratoy investigation of correlations (Pearson's) between changes in motor activity pattern before and after stimulation. **Aim 8:** Exploratory investigation of a correlation (Pearson's) between the level of the TMS motor threshold and the tolerated tACS level (lower level from 21 Hz and 40 Hz, based on subjective symptoms).
Study Duration	1 year
Participation Duration	2 weeks
Enrollment Duration	6 months

2. Introduction

2.1. Background Information

The decline of cognitive functions, especially memory, is a common complaint, both during the normal ageing process and in dementia (1): More than half of the population will probably face such symptoms in their second half of life.

Parallel to cognition, sleep architecture changes in older age, including a reduction of slow wave sleep (for a review see (2)). Given the importance of sleep for the consolidation of memory (for a review see (3) and (4)) and its role in reducing cellular waste (including amyloid, the pathological hallmark of Alzheimer's disease) via the glymphatic system (5), it seems plausible that a deterioration of sleep in older individuals partly explains a worsening of memory functions.

Despite the importance of the problem, there is still a lack of effective measures against cognitive decline in ageing individuals.

However, advances in neuroscience within the last few years have given us an increasingly detailed understanding of the oscillations of brain activity in neural circuitries associated with cognitive processes, offering possibilities of influencing them.

Transcranial alternating current stimulation (tACS) is an established, safe way to non-invasively modulate oscillations in neuronal networks and thus potentially restore and improve the associated cognitive functions (for a

recent review of applying tACS to improve cognition in various contexts see (6), for tACS to improve memory in healthy adults see (7)).

2.2. Scientific Rationale

This controlled cross-over pilot study will be used to prepare for a full-scale RCT in patients with mild cognitive impairment by investigating feasibility as well as acceptability and adherence of the study procedures in a sample of n=28 healthy elderly adults. Any effect on memory performance and sleep EEG will be documented but is not a requirement for the development of an RCT. We include people over 50 years of age because memory problems and neurodegenerative diseases (specifically Alzheimer's Disease) occur in the second half of life. The exclusion criteria are designed to ensure that people are not cognitively impaired by a disease of the brain or other impairment of bodily functions. In addition, we exclude contraindications to tACS.
The pilot study serves as a basis for the application of the technique in specific patient groups, in particular those with mild cognitive impairment.

3. Specific Aims

3.1. Aim 1

To establish acceptability of cognitive testing during tACS in healthy elderly particpants. The threshold is set to 80% of participants.

3.2. Aim 2

Evaluation of an effect of a single session of gamma-tACS on memory (a) and associative memory (b) performance compared with control tACS. The estimand of interest is the comparison of the individual results of the memory tests at the individual and at the group level (paired t-test or a non-parametric test, respectively). for delayed (immediately after stimulation) and ultra-delayed recall (after a night of sleep as well as after 5 days).

3.3. Aim 3

Feasibility (a) and acceptability (b) of an ambulatory single-channel EEG device. The estimands of interest are the proportion of participants with at least four hours of recording in the first night (a) and the proportion of participants with three nights of at least 4 hours of EEG data (b).

3.4. Aim 4

The comparison of specific sleep EEG features, i.e. sleep spindles (a) and slow wave sleep (b), which are both prominently associated with cognitive functioning, especially memory processes, after each stimulation condition. Comparison will be on individual and on group level (via t-test or a non-parametric test, respectively).

3.5. Aim 5

To investigate whether there is a correlation between the effects of tACS (versus control control tACS) on memory recall and memory-associated sleep EEG features (especially amount of sleep spindles and slow wave sleep) on an exploratory level.

3.6. Aim 6

To investigate the effect of a single session of gamma-tACS (compared to control control tACS) on different cognitive functions, i.e. a letter fluency task (a), executive functioning (Stroop Test (b), Trail Making Test (c)) and attention (Attentional Performance, TAP (d)). These tests will be performed during stimulation and on the day after stimulation, and comparison will be calculated on the individual and on the group level (paired t-test or non-parametric test, respectively). For (a) the number of correct words, of perseverations and of rule breaks will be calculated. For (b) and (c) time for test completion and errors will be calculated. For (d) percentile ranks for the following subtests will be calculated: Alertness, Go/NoGo, Divided Attention, Visual Scanning.

3.7 Aim 7

To investigate whether there is a correlation between the effects of stimulation (in general) and/or the specific type of stimulation (gamma versus control) on the motor activity level measured by an actigraphy watch.

3.8 Aim 8

To investigate if there is a relationship between the brain excitability derived from the subjective effect of tACS and the motor threshold measured by TMS.

Specific Aims with Measures and Aim-Specific Statistical Analysis Plans

Specific Aim	Outcomes Measures	Population Parameters to be Estimated ("Estimands")	Statistical Estimators
Aim 1: To investigate the feasibility of cognitive testing during tACS	Fraction of participants who complete both stimulation and cognitive testing sessions	Proportion of participants with completed stimulation- and testing-protocol	Observed proportion and 95% C.I.
Aim 2: Evaluate the effect of gamma-tACS on memory testing	Results of delayed ultra-delayed verbal memory and associative verbal memory recall (after stimulation, day after stimulation and 5 days after stimulation) for gamma versus control tACS	Memory recall in Rey Auditory Verbal Learning Test (a) and a word association test (b); amount of words and word-pairs remembered after stimulation, on day after stimulation and 5 days later will be recorded	Comparison between results after stimulation and sham on individual and group level (mean differences, regression coefficients, components of variance)
Aim 3: Investigate feasibility (a) and acceptability (b) of wearable device (single-channel EEG during the night)	Fraction of participants wearing the device on (a) the first and (b) all three nights planned according to the protocol.	Proportion of participants having worn the device during (a) the first and (b) all three nights for at least four hours each night	Observed proportion and 95% C.I.
Aim 4: Evaluate the effect of gamma-tACS on sleep spindles (a) and slow wave sleep (b) compared to sham stimulation	Amount of sleep spindles (a) and slow wave sleep (b) after tACS-stimulation and control stimulation.	Differences (individual level and means) of sleep spindle frequency (maximum number per hour) and slow wave activity (maximum minutes of slow wave sleep per hour)	Comparison between results after gamma-tACS and control tACS on individual and group level (mean differences, components of variance, regression coefficients)
Aim 5: To investigate whether there is a correlation between memory recall and sleep EEG	Memory and associative memory recall performance; EEG features (as for aim 4)	Correlation between changes in memory recall and sleep EEG (amount of spindles and slow wave sleep) for gamma- versus control tACS	Exploratory investigation of correlations (Pearson's) between stimulation induced changes in memory recall performance and sleep EEG features
Aim 6: To investigate the effect of stimulation on different cognitive functions, i.e. a letter fluency task (a), executive functioning (Stroop Test (b), Trail Making Test (c)) and attention	Effect of tACS and control stimulation on cognitive testing during and on the day after stimulation.	For (a) the number of correct words, of perseverations and of rule breaks will be calculated. For (b) and (c) time for test completion and errors will be calculated. For (d) percentile ranks for the following subtests will be calculated: Alertness, Go/NoGo, Divided Attention, Visual Scanning	Comparison between results after stimulation and sham on individual and group level (mean differences, regression coefficients, components of variance)

(Attentional Performance, TAP (d))			
Aim 7: To investigate whether there is a correlation between motor activity level and stimulation	Effect of stimulation on motor activity level	Correlation between changes in the amount of motor activity the day after stimulation versus the day before; (a) after stimulation in general and (b) after gamma- versus control stimulation.	Exploratory investigation of correlations (Pearson's) between the day before and after the (respective) stimulations.
Aim 7: To investigate whether there is a correlation between the TMS motor threshold and the tolerated tACS amplitude (based on subjective symptoms)	Association between TMS motor threshold and symptoms from tACS	Correlation between the level of TMS motor threshold and the highest tolerated tACS level (21Hz and 40Hz, lower value).	Exploratory investigations of correlations (Pearson's) between the measures (% and mA).

4. Study Design

This is a single-center prospective interventional pilot study with a controlled crossover-design. Each participant is exposed once to the intervention (tACS) and once to the control intervention (control tACS) to allow not only an interindividual but also an intraindividual comparison of outcomes under both conditions. Thus, each participant can serve as their own control.

First, an online prescreening (in REDCap) is performed, where basic eligibility information and informed consent for a screening by telephone is obtained. If, in the subsequent phone screen, inclusion/exclusion criteria are met and the participant provide verbal consent, a meeting on site (UNC Chapel Hill) will be arranged, where participants will sign informed consent and undergo baseline assessments (day 1). Afterwards, participants will receive the single-channel EEG for the following night (night 1). The next day (day 2), the intervention (gamma-tACS or control tACS) during cognitive testing will take place. During the following night (night 2) the EEG will be worn again for recording of sleep EEG at home. The follow-up cognitive testing will be scheduled for the day after (day 3), and again for the fifth day after stimulation (memory recall by telephone).
This procedure will be repeated for the respective other intervention (gamma-tACS or control tACS) one week (5-9 days) later. Each participant will be wearing an actigraph (accelerometer wristband) during the study period. Additionally, we will perform motor thresholding by TMS on day 2. Additionally, TMS motor thresholding will take place during day 2.

Centralized services are not used. All data (including baseline assessment, cognitive testing results) are stored in a REDCap database.

4.1. Treatment Design

Memory – as cognition in general – is based on synchronous oscillatory activity within and across neuronal networks. Neuronal network activity can be modified via non-invasive brain stimulation. Transcranial alternating current stimulation (tACS) is a well-established and safe form of non-invasive stimulation. Stimulation frequency and location is chosen to engage the neuronal networks of interest as a function of the psychological constructs investigated in a given study. Here, we will use gamma-frequency-tuned tACS targeting bilateral temporal lobes during cognitive testing including a verbal memory task, with ultra-delayed recall on the day after the stimulation and after five days.
There is evidence for the improvement of episodic memory through the use of tACS in the gamma-frequency range in healthy participants ((8), (9), (10), (11)). We favor the temporal lobes over other locations for stimulation because gamma band brain activity occurs in temporal neocortex and hippocampus, both during memory encoding in the awake state and during memory consolidation in slow wave sleep ((12), (13), (14)).

We decided for an alternative frequency (21 Hz) as control intervention in order to match the side effects associated with bilateral tempral stimulation. The tACS amplitude will be titrated individually (evaluation of side effects; start with 2mA peak-to-peak, -0.25mA until side effects tolerable for at least 1 hour, respective testing for 40 Hz and for 21 Hz, lower well-tolerated amplitude is being administered during tasks).

4.2. Experimental Design

This is a controlled cross-over study. Participants will be randomly allocated to the respective sequence (first tACS, then control stimulation – vice versa) via simple randomization, i.e. based on a single sequence of random sequence numbers generated by computer.

4.3. Measurement Design

Table 1. Variables of interest: their occasions of evaluation, their uses for the aims, their roles in the study

Variables within Domains	Scale[1]	Occasions[2]	Aims[3]	Main Roles
Identifiers				
Participant's unique ID	Nominal	All	All	Identifier
Intervention (A or B)*	Binary	E	All	Identifier
General Profile				
Age	Decimal yrs	S	All	Screening, covariate uses
Implanted device/hearing aid	Binary	S	All	Screening
Cardiac pacemaker	Binary	S	All	Screening
Willingness to abstain from alcohol ≥24h before visit	Binary	S	All	Screening
Known neurologic diseases	Binary	S	All	Screening
Known psychiatric diseases	Binary	S	All	Screening
Known other medical conditions	Binary	S	All	Screening
Brain surgery in the past	Binary	S	All	Screening
Night shifts, bed after midnight	Binary	S	All	Screening
Use of illegal drugs	Binary	S	All	Screening
Sex	Categorical	0	All	Covariate uses
Racial/ethnic categories	Categorical	0	All	Covariate uses
Education	Categorical	0	All	Covariate uses
Living conditions	Categorical	0	All	Covariate uses
Work status	Categorical	0	All	Covariate uses
Clubs/social organizations	Binary	0	All	Covariate uses
Sports	Binary, categorical	0	All	Covariate uses
Social contacts	Binary, categorical	0	All	Covariate uses
Media consumption	Categorical	0	All	Covariate uses
Concomitant medications list	Nominal	0	All	Covariate uses

Caffeine consumption	Binary, nominal, dL	0	All	Covariate uses
Alcohol consumption	Binary, nominal, dL	0	All	Covariate uses
Smoker	Binary, yrs, cig/d	0	All	Covariate uses
Questionnaires				
Sleep Quality Assessment PSQI (15)	Ordinal 0-21	0	All	Screening
Patient Health Questionnaire-9 (16)	Ordinal 0-27	0	All	Screening
General Anxiety Disorder-7 (17)	Ordinal 0-21	0	All	Screening
Alcohol Use Disorders Identification Test (1-3) (18)	Ordinal 0-18	S	All	Screening
AD8 Dementia Screening (19)	Ordinal 0-8	0	All	Screening
Montreal Cognitive Assessment (20)	Ordinal 0-30	0	All	Screening
Epilepsy Screening (21)	Binary	0	All	Screening
Handedness Questionnaire (22)	Laterality Index	0	All	Covariate uses
Behavioral Inhibition and Behavioral Activation Self Report Scales (BIS/BAS) (23)	Ordinal, 4 subscales: BIS (7 to 28) BAS drive (4-16) BAS fun-seeking (4-16) BAS reward (7-20)	0	All	Covariate uses
Basic Sleep Habits	hh:mm; hours/min	0	All	Covariate uses
Stimulation conditions				
Epileptiform activity in EEG	Binary	0	All	Early termination of study participation
Karolinska Sleepiness Scale (24)	Ordinal 1-10	D1A, D2A, D1B, D2B	Aim 2	Covariate use
PANAS (Positive and Negative Affect Schedule) (25)	Ordinal 20-100	D1A, D2A, D1B, D2B	Aim 2	Covariate use
Sleep quality night before	Categorical (nominal)	D1A, D2A, D1B, D2B	Aim 2	Covariate use
Cognitive testing				
Completed	Binary	D1A, D2A, D1B, D2B	Aim 1	Primary outcome
Delayed and ultra-delayed recall from Rey Auditory Verbal Learning Test (RAVLT) (26) and from word	Ratio (number of correct words); number of correct words for trials 1-5 (=learning), for trial 6 (“interference”) and for trial 7 (“recall”);	D1A, D2A, PA, D1B, D2B, PB	Aim 2, Aim 5	Primary outcome

association test; RAVLT encoding	number of confabulations and perseverations in total)			
Time from encoding of RAVLT and word association test to delayed and ultra-delayed recall	Interval (hh:mm)	D1A, D2A, PA, D1B, D2B, BP	Aim 2	Covariate use
Time from encoding of word association test to delayed and ultra-delayed recall	Interval (hh:mm)	D1A, D2A, PA, D1B, D2B, BP	Aim 2	Covariate use
RAVLT	Ratio (number of correct words for trials 1-5 (=learning), for trial 6 (“interference”) and for trial 7 (“recall”); number of confabulations and perseverations in total)	D1A, D1B	Aim 2	Covariate use
Word association test	Ratio (number of correct words for trials 1-4)	D1A, D1B	Aim 2	Covariate use
Letter Fluency (28)	Ratio (number of correct words, number of perseverations and number of rule breaks)	D1A, D2A, D1B, D2B	Aim 6	Secondary outcome
Stroop Color and Word Test (29)	Interval (sec), ratio (errors)	D1A, D2A, D1B, D2B	Aim 6	Secondary outcome
Trail Making Test A and B (30)	Interval (sec), ratio (errors)	D1A, D2A, D1B, D2B	Aim 6	Secondary outcome
Test of Attentional Performance (TAP) (31)	Interval, ratio (Alertness: Percentile rank of median reaction time for correct responses, percentile rank of standard deviation; Go/NoGo: Percentile rank of median reaction time for correct responses, percentile rank of standard deviation, percentile rank of errors; Divided Attention: Percentile rank of median reaction time for correct responses, percentile rank of standard deviation, percentile rank of errors, percentile rank of omissions; Visual Scanning: Percentile rank of median of critical and non-critical, respective standard deviation, omissions)	D1A, D2A, D1B, D2B	Aim 6	Secondary outcome
Sleep EEG				
First night with ≥4 hours of recording	Binary (hh:mm)	N1	Aim 3	Primary outcome; early termination of study participation if <4 hours
EEG worn for ≥4h hours in 3/3 nights	Binary (nights)	N1, N2A, N2B	Aim 3	Primary outcome

Sleep latency	Minutes	N1, N2A, N2B	Aim 4, Aim 5	Covariate use
Sleep efficiency	%	N1, N2A, N2B	Aim 4, Aim 5	Covriate use
Sleep spindles, slow wave sleep	Amount/hour	N1, N2A, N2B	Aim 4, Aim 5	Primary outcome
Subjective tolerability of headband	Binary, nominal	N1, N2A, N2B	Aim 3	Exploratory use
Actigraphy				
Motor activity/24 hours, resting time for sleep	%, hours	N2A, D2A, N2B, D2B	Aim 7	Exploratory outcome
Thresholding				
TMS motor threshold	% MSO (maximal stimulator output)	D2A	Aim 8	Exploratory outcome
tACS thresholding	mA (lower value of 21 Hz and 40 Hz)	D2A	Aim 8	Exploratory outcome
Safety Monitoring				
AEs and SAEs documentation	Events	All	All	Safety monitoring

[1] Units of measurement or the scale.
[2] Occasions of evaluation or retrieval:
S = Screening
0 = Enrollment and Baseline
N1 = Night 1
D1A = Day 1 in Intervention A
N2A = Night 2 in Intervention A
D2A = Day 2 in Intervention A
PA = Phonecall in Intervention A
D1B = Day 1 in Intervention B
N2B = Night 2 in Intervention B
D2B = Day 2 in Intervention B
PB = Phonecall in Intervention B

[3] The specific aims in which the variable will play a role in data analyses.
[4] Uses: assess medication adherence, mediation analyses, and exploratory analyses

*Intervention:
A = tACS
B = control stimulation

5. Study Participants

5.1. Numbers of Participants

5.1.1. Number to be screened: N ≤ 100

5.1.2. Number to be enrolled: N ≤ 50

The sample size is 28 participants in the final dataset, i.e. the participants who completed all the visits for the primary outcomes, i.e. Visits 1, 2, 3, 4, 5, 6, 7 (including the three nights with EEG). However, to ensure that 28 participants complete the experiment, we conservatively estimate to screen 100 participants and to enroll 50 participants as a ceiling for the sake of IRB approval. The motivation for the sample size of 28 participants is the following power analysis: power analysis: dz = 0.5, 1-beta = 0.80, alpha = 0.05. Participants who drop-out or have missing data will be replaced.
Recruitment takes place in the general population.

5.2.Eligibility Criteria

Participants have to complete a set of questions (in REDCap) during the screening process. They will be instructed to consume no alcohol and no more caffeine than usual 24 hours before each study visit.

5.2.1. Inclusion Criteria

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

- >50 years old

5.2.1. Exclusion Criteria

Any individual who meets one or more of the following criteria will be excluded from participation:

- Implanted device or metal in head (including cochlear implant or other hearing aid), cardiac pacemaker or any other powered medical device
- Known neurological disease from history (epilepsy, sleep disorder (insomnia, sleep apnea, restless legs syndrome, parasomnia), stroke or transitory ischemic attack, cognitive impairment, neurodegenerative disease (for example Alzheimer's disease, Parkinson's disease or amyotrophic lateral sclerosis), immune-mediated disease of the central nervous system, chronic infectious brain disease, brain tumor, traumatic brain injury with loss of consciousness and/or intracranial bleeding, chronic pain with the need for daily analgesic use)
- Positive screening for epilepsy (questionnaire)
- Pathological Montreal Cognitive Assessment (MoCA <26/30 points)
- Brain surgery in the past (lifetime)
- Known psychiatric disorder from history (schizophrenia (lifetime), obsessive compulsive disorder (OCD; lifetime), borderline personality disorder (lifetime), anxiety disorder (lifetime), bipolar disorder (lifetime), psychosis (lifetime), eating disorder (lifetime), depression (within the last three months)
- Positive screening for anxiety disorder (GAD-7 ≥10/21 points) or positive screening for depression (PHQ-9 ≥5/27 points)
- Known other relevant medical condition from history(moderate to severe chronic obstructive pulmonary disease (COPD), abnormal kidney function (defined as estimated Glomerular Filtration Rate <60ml/min), current liver disease (defined as hepatitis and/or liver cirrhosis), cancer, diabetes mellitus, cardiac disease (heart failure, myocardial infarct, atrial fibrillation and revascularization – all within the last three months)
- Working in night shifts or going to bed after midnight on 3 or more nights per week
- Positive screening for sleep disorder (PSQI >5/21 points)

- Psychotropic treatment or illegal drugs (including cannabis) within the last three months
- Indication for alcohol use disorder: AUDIT score (Alcohol Use Disorders Identification Test; screening for unhealthy alcohol use) ≥7 for females and for males ≥ 65 years or ≥8 for males <65 years
- Not willing to abstain alcohol at least 24 hours before each study visit
- Women who want to become pregnant, are pregnant, plant to seek or are seeking fertility treatment

5.3. Enrollment/Selection Strategies

5.3.1. Prospective Recruitment

We will advertise the study directly to the public on websites such as ClinicalTrials.gov, studypages.com, Research For Me, frohlichlab.org and Carolinaneurostimulation.org. We will have contact information and a summary of the clinical trial posted on the Frohlich Lab Facebook and Twitter pages. We will also be launching a Facebook or Instagram advertisement to identify potential participants. Furthermore, we will also be using the UNC Mass email and department listserv to send out an email containing the advertisement. In addition, we will post fliers senior living communities. We have previously recruited about 50 participants from an identical population within 3 months.

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

The advertisement will include a link to a brief prescreening survey on REDCap to help identify participants. Interested individuals can then either visit the specified website and complete an online screening survey on REDCap or call or email to register for a telephone prescreening. All participant identifiers will be stored in REDCap and password encrypted tables stored on UNC servers until recruitment is over. When recruitment is over, all patients who do not consent or are not eligible for participation in the study will have their responses permanently deleted.

Our retention strategy includes a payment schedule of two times $50 per participants. Thus, completion of the study will result in a financial compensation of $100.

5.3.2. Screen Failures

In the case that a participant enrolls in the trial and the screening reveals that they do not meet study criteria, the study personnel completing the interviewing process will clearly explain why the participant does not meet criteria. If a time-sensitive criterion is not met, re-screening may occur at a later time. In the worst case, we expect only ~25% of those interested to be eligible.

5.4.Strategies for Retention

Due to the low time burden (five study visits on site) at short intervals (maximum two weeks), we expect a low drop-out rate. Nevertheless, we are capturing data on possible reasons in case of discontinuation.

5.5. Matching and Stratification

Not applicable.

5.6. Randomization and Concealment

Participants will be assigned to active stimulation first or sham stimulation first using simple randomization.

5.7. Blinding

This is a double-blind study. Both participants and research staff will be blinded to the stimulation condition.

6. Treatment Design: Procedures

Description

Transcranial alternating current stimulation (tACS)

Participants will be stimulated with the commercial, CE-certified Neuroconn multiple channel (MC) stimulator. The use of this device routinely received a non-significant risk (NSR) designation on review by the full UNC IRB. The NeuroConn device description is as follows: The DC-STIMULATOR MC is a CE-certified medical device for conducting non-invasive transcranial current stimulation in humans. The DC-STIMULATOR MC is a micro-processor-controlled current source. It meets the highest safety standards thanks to (hardware- and software-based) multistage monitoring of the current path. By continuously monitoring electrode impedance it can detect insufficient contact with the skin and automatically terminate stimulation, maximizing patient safety. The device includes a digital display with various stimulation modes to be selected and stimulation parameters such as current strength, duration, fade-in and fade-out to be set.

DC-STIMULATOR MC features:

- 4 programmable, micro-processor-controlled constant current sources using 25 ndependent channels (optional: 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 standard modes – tDCS (continuous stimulation) – pulse (cyclical stimulation activation/deactivation) – sinus (sinus wave) – noise (normally distributed)
- Current strength and curve forms adjustable up to ±4,000 µA, AC current strength adjustable up to 8,000 µA (peak-to-peak)
- Frequencies adjustable up to 1,000 Hz, phase freely adjustable

Electroencephalography (EEG):

A high-density EEG net (128 electrodes including electrooculography electrodes; EGI, Inc.). Continuous qEEG recording will be performed. Sampling rate during the continuous recording will be 1 kHz with Cz as the reference and a channel between Cz and Pz as ground, using an EGI system with SDK AmpServer Pro (Geodesic, Eugene, Oregon).

Transcranial magnet stimulation (TMS) motor thresholding:

Additionally, TMS motor thresholding (M. interosseus dorsalis I, with EMG electrodes) will be performed, as there is evidence suggesting a relationship between the effect of tACS and brain excitability measured with single pulse TMS. We therefore measure cortical excitability by performing motor thresholding using single pulse TMS (transcranial magnetic stimulation) during Visit 2. A MagVenture MagPro X100 TMS device will be used.

Dosing and Administration

The research team will first measure each participant's head using the 10-20 system to determine the electrode locations. Participants will then be fitted with the 4 electrodes for stimulation: two 4.5x4.5cm electrodes placed over T7/P7 and T8/P8 (10/20-EEG-system). Electrodes will be carbon rubber, with Ten20 conductive paste applied.

For gamma-tACS 40 Hz will be applied (“natural target value”, for a review see (33)). We have decided to apply control stimulation instead of sham stimulation as we have been able to demonstrate visual side effects with bitemporal asynchronous stimulation (i.e. synchronized within one hemisphere) which differ according to stimulation frequency (phosphenes with higher frequencies, sensation of movements of the environment with lower frequencies). Hence, 21 Hz tACS will be applied for control stimulation (reasoning: no known specific effects on temporal lobe; close to gamma-stimulation and hence similar concerning side effects; not a harmonic frequency to 40 Hz). An individual current amplitude will be determined before stimulation (for 21 Hz and 40 Hz; ramp-on 40s, continuous stimulation 20s; from 2mA peak-to-peak in steps of 0.25mA until “symptoms well tolerable for at least

one hour". The lower of the two current amplitudes from 40 Hz and 21 Hz will be applied for both stimulation conditions).

For both stimulation sessions, a ramp-on and ramp-off time of 40s will be applied.

Researchers will monitor participants during stimulation. Personnel will be thoroughly trained and have trainings documented on the transcranial stimulation device and will be present during all stimulation sessions. Co-investigator Andrea Seiler MD is a Swiss board-certified neurologist who will serve as supervisor for the stimulation study visits.

During stimulation, the participants will perform the following cognitive tests presented on the computer screen or on paper: RAVLT (Rey Auditory Verbal Learning Test) (26), a word association test (8 word-pairs), Letter Fluency (28), Stroop Color and Word Test (29), Trail Making Test A and B (30), TAP (Test of Attentional Performance) (31). The stimulator will be triggered by remote control from the experiment script and will administer stimulation during each single test. After each block of stimulation, an eyes-open resting state EEG will be performed. All stimulation involves 20 seconds of ramp-in time and 20 seconds of ramp-out time. The total duration of the stimulation and testing is approximately 40 minutes.

7. Schedule of Activities and Procedures

7.1.Table of Events

Procedure		Visit 0 screening (online/phone)	Visit 1 Baseline (from day after Visit 0 to +14 days)	Visit 2 (day after Visit 1)	Visit 3 (day after Visit 2)	Visit 4 (telephone; 5 days after Visit 2)	Visit 5 (6-9 days after Visit 2)	Visit 6 (day after Visit 5)	Visit 7 (telephone; 5 days after Visit 5)
Recruitment	Informed consent	X (oral)	X (written)						
	Eligibility assessments	X	X						
	Enrollment and randomization		X						
Eligibility assessments	At least 4 hours of initial sleep-EEG			x					
	No epileptiform discharges in baseline-EEG		x				x		
	No psychotropic treatment/illegal drugs within the last 3 months, no alcohol within the last 24 hours			x	x		x	x	
Intervention	(Control) Stimulation			X			X		
	Cognitive testing[1]			X	X	X (only verbal memory and associative verbal memory recall)	X	X	X (only verbal and associative verbal memory recall)
	Sleep-EEG[2]		X (next night)	X (next night)			X (next night)		
	Actigraphy		X	X	X	X	X	X	
	TMS Motor Thresholding			X					
Patient Reported Outcome	Subjective tolerability of sleep EEG			X	X			X	
	Sleepiness (Karolinska Scale)			X	X		X	X	
	Sleep quality			X	X		X	X	
	Affect (PANAS)			X	X		X	X	
Safety Monitoring	Assessment of AEs			X	X	X	X	X	X

[1] Standardized results of: RAVLT (Rey Auditory Verbal Learning Test) (26), word association test, Letter Fluency (28), Stroop Color and Word Test (29), Trail Making Test A and B (30), TAP (Test of Attentional Performance) (31).
[2] Device worn (yes/no), hours of recording, sleep latency, sleep efficiency, amount of sleep spindles and slow wave sleep.

7.2. Screening

The pre-screening can be performed via REDCap (questions 1, 2, 3, 4); the whole screening (questions 1-12) is performed via telephone. Result of the screening procedure: eligible or not eligible.
The following questions will be asked:
1. Are you at least fifty years old? If not, then not eligible.
2. Do you have an implanted device or metal in your head (including a cochlear implant), or do you have hearing aids? If yes, then not eligible.
3. Do you have a cardiac pacemaker? If yes, then not eligible.
4. Alcohol Use Disorders Identification Test (AUDIT) 1-3: Gender: Woman / man / transgender / non-binary / prefer not to respond. Age: <65 years / ≥65 years? How often do you have a drink containing alcohol? How many drinks containing alcohol do you have on a typical day when you are drinking? How often do you have 4 (for females) or 5 (for males) – or more – drinks on one occasion? Cutoff (i.e. not eligible): ≥7 for females and for males ≥ 65 years; ≥8 for males <65 years.
5. Are you willing to abstain alcohol at least 24 hours before each study visit? If no, then not eligible.
6. Have you ever been told by a healthcare provider that you have one of the following neurologic diseases: Epilepsy, sleep disorder (insomnia, sleep apnea, restless legs syndrome, parasomnia), stroke or transitory ischemic attack, cognitive impairment, neurodegenerative disease (for example Alzheimer's disease, Parkinson's disease or amyotrophic lateral sclerosis), immune-mediated disease of the central nervous system, chronic infectious brain disease, brain tumor, traumatic brain injury with loss of consciousness and/or intracranial bleeding, chronic pain with the need for daily analgesic use? If yes, then not eligible.
7. Did you ever have brain surgery? If yes, then not eligible.
8. Have you been told by a healthcare provider that you have one of the following psychiatric diseases (in your lifetime or during the last three months): Schizophrenia (in your lifetime), obsessive compulsive disorder (OCD; in your lifetime), borderline personality disorder (in your lifetime), anxiety disorder (in your lifetime), bipolar disorder (in your lifetime), psychosis (in your lifetime), eating disorder (in your lifetime), depression (during the last three months)? If yes, then not eligible.
9. Have you ever been told by a healthcare professional that you have one of the following medical conditions: Moderate to severe chronic obstructive pulmonary disease (COPD), abnormal kidney function (defined as estimated Glomerular Filtration Rate <60ml/min), current liver disease (defined as hepatitis and/or liver cirrhosis), cancer, diabetes mellitus, cardiac disease (heart failure, myocardial infarct, atrial fibrillation and revascularization – all within the last three months)? If yes, then not eligible.
10. Do you work night shifts (last three months)? If yes, then not eligible.
11. Do you usually go to bed after midnight (last three months)? If yes, then not eligible.
12. Have you used psychotropic treatment or illegal drugs (including cannabis) within the last three months? If yes, then not eligible.

If online pre-screening is successfully completed and the participant appears eligible, the following message will appear: Please leave name, email, phone number and preferred time to be called by phone.

The following information will be provided by telephone:
1. A brief summary of the study and its objectives.
2. Instructions for the meeting at the lab:
    2.1 In the 24 hours before the visit: Please do not drink alcohol and do not drink more caffeine than you usually do.
    2.2 Please bring a list of all medications you regularly take (including those not prescribed by a healthcare professional).

If not yet the case, the online questions will be completed by phone.

There may be up to 28 days between (pre-)screening and enrollment.

7.3. Enrollment

Visits 0 and 1

The following procedures will occur:
1. Signing of informed consent
2. Completion of demographics and supplementary questions (mainly providing information about the cognitive state): Sex, racial/ethnic categories, education, living conditions, work status, clubs/social organizations, sports, social contacts, media consumption, concomitant medications list, caffeine consumption, alcohol consumption, smoking status.
3. Specific questionnaires to exclude patients with indications for a sleep disorder, depression, anxiety disorder, cognitive impairment, epilepsy:
3.1 Sleep Quality Assessment PSQI (15): Screening for sleep disorders. Result: 0-21 points. Exclusion if >5 points.
3.2 Patient Health Questionnaire-9 (16): Screening for depression. Result: 0-27 points. Exclusion if ≥5 points.
3.3. General Anxiety Disorder-7 (17): Screening for anxiety disorder. Result: 0-21 points. Exclusion if ≥10 points.
3.4. Montreal Cognitive Assessment (MoCA): Screening for dementia. Result: 0-30. Exclusion if <26.
4. Complementary questionnaires to complete patient profile:
4.1 Handedness Questionnaire (22)
4.2 Behavioral Inhibition and Behavioral Activation Self Report Scales (BIS/BAS) (23): These scales are used to monitor the perceived sensitivity to reward and punishment. BIS/BAS is broken into four sub-scores: BIS (7 to 28), BAS drive (4-16), BAS fun-seeking (4-16), and BAS reward (7-20).
4.3 Basic sleep habits (bedtime, sleep latency, wakefulness during the night, waking time, total amount of sleep on average, optimal total amount of sleep)
5. The following instructions will be given for the night and following day:
851 How to use the EEG-headband the night before visit 2 (oral and written instructions, including sleep diary); how to use the actigraphy watch.
5.2 To not consume more caffeine than usually. To please not drink alcohol.
6. The participant will be randomized to the respective intervention for visit 2 (either gamma-tACS or first control stimulation).

7.4. Study Visits

Visit 2 (day after Visit 1; afternoon/early evening, i.e. start between 12pm and 6pm)

1. Questioning whether there has been any use of alcohol, psychotropic substances or illicit drugs during the past 24 hours. If yes, study participation is terminated.
2. Questions to assess current sleepiness and mood, as this may affect the results of further testing:
    2.1 Questions for sleep quality the night before
    2.2 Karolinska Sleepiness Scale (24)
    2.3 Positive And Negative Affect Schedule (PANAS) (25)
3. Motor thresholding by TMS.
4. Thresholding individual tACS amplitude.
5. Gamma-tACS or control stimulation (according to randomization) during the following cognitive tests, presented on a computer screen (2 minutes of resting EEG without stimulation after each test; total duration approximately 40 minutes):
    5.1 Rey Auditory Verbal Learning Test (RAVLT, version 1) (26)
    5.2 Letter Fluency; letters F, A, S (28)
    5.3 Stroop Color and Word Test (29)
    5.4 Trail Making Test A and B (30)

5.5 Word association test; version 1
5.6 Four subtests of the Test of Attentional Performance (TAP) (31): Alertness, Go/Nogo, Divided Attention, Visual Scanning
5.7 RAVLT delayed recall

6. Evaluation of side effects with open question ("Did you feel anything particular or note anything special during this session?")
7. The participant will be given the sleep diary for the second night with the EEG headband.

Visit 3 (day after Visit 2; morning, i.e. start between 8am and 12pm)

1. Questioning whether there has been any use of alcohol, psychotropic substances or illicit drugs during the past 24 hours. If yes, the participant is excluded.
2. Questions to assess current sleepiness and mood, as this may affect the results of further testing:
    3.1 Questions for sleep quality the night before
    3.2 Karolinska Sleepiness Scale
    3.3 Positive And Negative Affect Schedule (PANAS)
3. Cognitive follow-up testing
    4.1 RAVLT "ultra-delayed" recall; version 1
    4.2 Letter fluency; letters B, H, R
    4.3 Stroop Color and Word Test (SCWT)
    4.4 Trail Making Test Part A and B
    4.5 Word association recall; version 1
    4.6 Four subtests of the Test of Attentional Performance (TAP): Alertness, Go/NoGo, Divided Attention, Visual Scanning

Visit 4 (telephone; 5 days after Visit 2)

Performed by telephone.

1. Assessment of adverse events.
2. RAVLT "ultra-delayed" recall; version 1.
3. Word association recall; version 1.

Visit 5 (6-9 days after Visit 2; afternoon/early evening, i.e. start between 12pm and 6pm)

The day before: reminder (e-mail/SMS/phone call, depending on the participant's preference) of appointment on the following day - including request to abstain from alcohol and drugs as well as from excessive caffeine consumption.

1. Questioning whether there has been any use of alcohol, psychotropic substances or illicit drugs during the past 24 hours. If yes, the participant is excluded.
2. Questioning if there is a new diagnosis. If yes, the participant will be excluded if the diagnosis meets exclusion criteria.
3. Questions to assess current sleepiness and mood, as this may affect the results of further testing:
    3.1 Questions for sleep quality the night before
    3.2 Karolinska Sleepiness Scale
    3.3 Positive And Negative Affect Schedule (PANAS)
4. Gamma-tACS or controlstimulation (according to randomization) during the following cognitive tests, presented on a computer screen (2 minutes of resting EEG without stimulation after each test):
    4.1 Rey Auditory Verbal Learning Test (RAVLT, version 2)
    4.2 Letter Fluency; letters F, A, S

4.3 Stroop Color and Word Test
4.4 Trail Making Test A and B
4.5. Word association test; version 2
4.6 Four subtests of the Test of Attentional Performance (TAP): Alertness, Go/NoGo, Divided Attention, Visual Scanning
4.7 RAVLT delayed recall; version 2

5. Evaluation of side effects with open question ("Did you feel anything particular or note anything special during this session?")
6. The participant will be given the sleep diary for the third night with the EEG headband.

Visit 6 (day after Visit 5; morning, i.e. start between 8am and 12pm)
1. The participant brings back the EEG-headband (used the night before). Data quality check, review of sleep diary.
2. Questioning whether there has been any use of alcohol, psychotropic substances or illicit drugs during the past 24 hours. If yes, the participant is excluded.
3. Questions to assess current sleepiness and mood, as this may affect the results of further testing:
   3.1 Questions for sleep quality the night before
   3.2 Karolinska Sleepiness Scale
   3.3 Positive And Negative Affect Schedule (PANAS)
4. Cognitive follow-up testing
   4.1 RAVLT "ultra-delayed" recall; version 2
   4.2 Letter fluency; letters B, H, R
   4.3 Stroop Color and Word Test (SCWT)
   4.4 Trail Making Test Part A and B
   4.5 Word association recall; version 2
   4.6 Four subtests of the Test of Attentional Performance (TAP): Alertness, Go/NoGo, Divided Attention, Visual Scanning

Visit 7 (telephone; 5 days after Visit 5)
Performed by telephone.
1. Assessment of adverse events.
2. RAVLT "ultra-delayed" recall.
3. Word association recall; version 2.

7.5. Early Discontinuations

Data to be Collected

If the study participation has to be terminated early by the investigators (due to safety concerns), or if a participant chooses to withdraw from the study, including the case of not showing up for scheduled visits on site (Visits 1, 2, 3, 5, 6), the data collected to date will not be included for the cognitive and sleep-EEG outcomes and the participant will be replaced. However, early discontinuation will be included in the data set for the feasibility outcomes as appropriate. Visits 4 and 7 are not mandatory for study completion.

Criteria for Intervention Discontinuation

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

- Any clinical adverse event (AE), intercurrent illness or other medical condition, or situation occurs such that continued participation in the study would not be in the best interest of the participant.

- The participant meets any exclusion criteria (either newly developed or not previously recognized) or a reason for immediate early termination of participation.

7.6. Enrollees May Drop Out

Participants may voluntarily withdraw from participation at any time, for any reason, with no penalty or loss of rights. In case of no-show, an attempt will be made to contact the participant (by mail or phone). The reasons for drop-out and missing data will be documented in the database.

8. Statistical Analysis Plans

8.1. Strategies that Apply to all the Aims

All testing described below assumes a significance threshold of p = 0.05. Analyses will be deemed to be statistically significant if the p-value is less than this threshold. An analysis that does not exceed this threshold will be considered inconclusive. The proposed statistical analysis strategy acknowledges that no p-value can reveal the plausibility, presence, truth, or importance of an association or effect.

There may be additional covariates included in the analysis. Data will be assessed for normality and, if deemed necessary, corrective procedures will be applied (e.g., log normalization). This correction will be applied upon consideration of the variables themselves, and not based on the result of the intended analysis.

8.2 Sample Description

The sample will be described using the following parameters: Age, sex, racial/ethnic categories, education, living conditions, work status, clubs/social organizations, sports, social contacts, media consumption, caffeine and alcohol consumption, smoking status. Whenever feasible, graphical methods will be used to describe the sample. Continuous data will be described using means, standard deviations, and confidence intervals, while categorical data will be described using counts/percentages.

8.3 Aim-Specific Plans

Plans for Aim 1.

a) The proportion of participants in which stimulation and cognitive testing could be completed will be calculated after both stimulation sessions, i.e. visit 2 and visit 4 (percentage and 95% confidence interval).

Plans for Aim 2.

To evaluate the effect of gamma-tACS on cognitive testing.

a) Primary outcome: Ultra-delayed verbal memory recall (i.e. amount of words and word associations remembered the day after encoding the RAVLT) after gamma-tACS compared to after control stimulation, intra- and interindividually.
We will perform a two-way or one-way repeated measure ANCOVA with time (baseline and post-treatment) and treatment (control vs gamma-tACS) as within-subjects factors, and the order in which tACS will be applied (tACS-sham or control tACS) as covariate. Post-hoc tests will be calculated after Bonferroni correction for multiple comparisons.

Null hypothesis: There is no difference between tACS and control stimulation for ultra-delayed memory recall one day after stimulation. Alternative hypothesis: Memory recall is better after gamma-tACS than after control stimulation.

b) Secondary outcomes: Verbal and associative learning (immediate and delayed recall, recall after 5 days) and executive functions (results of Letter Fluency, Stroop Test, Trail Making Test, Tests of Attentional Performance), comparison intra- and interindividually, after gamma-tACS and control stimulation (comparison of difference

between visit 1-visit 2 and visit 3-visit 4; comparison of absolute results of visit 1 versus visit 3 and visit 2 versus visit 4; statistical tests and covariates as in a)).
Hypotheses are the same as for the primary outcome.

Plans for Aim 3.

To investigate the feasibility and adherence to wearable device (single-channel EEG during the night).

a) The proportion (percentage and 95% confidence interval) of participants with at least four hours of EEG recording in the first night.

b) The proportion (percentage and 95% confidence interval) of participants with at least four hours of recorded nocturnal data for each of all three nights.

Plans for Aim 4.

To evaluate the effect of gamma-tACS compared to control stimulation on sleep EEG. Differences (individual level and group level, statistical tests as mentioned above) of sleep latency (minutes from lights out to sleep onset), sleep efficiency (sleep duration in relation to time spent in bed), sleep spindle frequency (maximum number per hour) and slow wave activity (maximum minutes of slow wave sleep per hour).
The statistical method will be the same as for Aim 2.
Null hypothesis: Sleep EEG does not change after gamma-tACS compared to the night after control stimulation.
Alternative hypothesis: There is a higher amount of sleep spindles and/or slow wave sleep after gamma-tACS (compared to the control).

Plans for Aim 5.

To investigate whether there is a correlation (Pearson's) between the stimulation-induced changes in cognitive testing scores and sleep EEG features after gamma-tACS versus after control stimulation (on individual and group level). This is an exploratory analysis.

Plans for Aim 6.

Comparison of cognitive test results after stimulation and sham on individual and group level using ANCOVA (see CT.gov). This is an exploratory analysis.

Plans for Aim 7. To investigate whether there is a correlation (Pearson's) between the stimulation-induced changes in motor activity features (according to the wristband accelerometer results) after stimulation in general and after gamma-tACS versus after control stimulation (on individual and group level). This is an exploratory analysis.

Plans for Aim 8.

To investigate whether there is a correlation (Pearson's) between the motor threshold (TMS) and the subjective symptoms during tACS. This is an exploratory analysis.

8.4 Planned Interim Analyses

No interim analyses will be performed.

9. Sample Size Rationale

We plan to recruit 28 healthy adults in this feasibility study (primary outcome). The sample size was computed such that the study has 95% power (alpha = 0.05) to reject the null hypothesis that 50% of the population of interest can complete the study procedures for an effect size of g = 0.3, meaning that if at least 80% of the study sample complete the procedures, feasibility in the population of at least 50% is established.

10. Data Capture and Database Management

10.1. Software for Data Capture

Data will be entered into a data capture system provided by TraCS Clinical Research Data Management Service (REDCap). REDCap is a 21 CFR Part 11-compliant data capture system provided by the NC TraCS Institute at UNC. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate.

10.2. Responsibilities for Data Capture and Database Management

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

Hardcopies of the study visit worksheets will be provided for use as source document worksheets for recording data for each participant enrolled in the study. 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) will be entered into REDCap. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents. Trained research personnel (including the co-investigator) will have complete access to the REDCap system, while the principal investigator will have read-only ability.

10.3. Study Records Retention

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

11. Collection and Management of Tissue Specimens

Not applicable.

12. Safety Monitoring and Management

12.1. Risk / Benefit Assessment

Potential Risks:

Risk of Confidentiality Breach: To avoid the uncommon event breaches in confidentiality, study documents that contain personal information, including the informed consent document, will be kept in locked filing capinets in locked rooms. The document that links study ID numbers to personal identifying information will be a password-protected spreadsheet. All data is stored in locked cabinets inside locked offices; electronic data will be stored only on password-protected computers, and data encryption methods will be used during communication between investigators. Interviews will be conducted over the HIPAA approved Zoom account. Only study personnel will have access to the data. All study staff participate in annual human 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. This uncommon risk is necessary in order to assess for relevant preexisting disorders. Participants will be assured upon intake that only study personnel will see any clinical ratings and that self-identifying information will not be collected alongside HIPAA protected information.

Risk of Injury and Discomfort:

The side effects of tACS are mild and transient; in fact, low intensity transcranial current stimulation, such as tACS, has been used for several years without any report of serious side effects. Furthermore, this stimulation mode has nothing to do with electroconvulsive therapy that applies many orders of magnitude higher stimulation current. Rather, transcranial current stimulation is so weak that it does not cause super-threshold activation of neurons.

TACS does have some mild side effects, such as transient tingling, burning, pulsing sensation, or itching under the electrode sites (common). In a previous tACS trial we conducted, participants from all three groups of stimulation (two stimulation conditions, one was an active placebo group) reported either absent or mild side effects, and there was no difference between the groups with the exception of “flickering lights” (or phosphenes, $p = 0.014$). To monitor these mild side effects, we will be administering a stimulation questionnaire after each stimulation session to determine whether these effects were experienced and at what intensity. Research personnel is present during the full experiment. There have also been infrequent reports of reduced ability to focus, change in cognition, altered mood, headaches, and sleepiness. There is no evidence available that indicates that these potential side-effects are associated with stimulation versus study participation in general. In our previous depression work, we found no signal that supports an associated between any of these side-effects and the administration of tACS. Both the application of the tACS and the EEG electrodes can cause mild inconvenience such as mild headaches from the pressure of applying the electrodes.

The motor thresholding procedure that uses TMS has been used without reports of any serious side-effects. Some subjects report muscle twitching during stimulation and sometimes a headache (common, <50% incidence), but these possible effects are transient and do not persist after we stop stimulation. The transcranial stimulator used in this study was cleared by UNC Hospital Medical Engineering, the in-house engineering team of UNC hospitals that test the safety of medical devices. This TMS device has been cleared by the FDA for some indications, such as Major Depressive Disorder in patients, but it has not been evaluated by the FDA for this study. To mitigate

uncomfortable side effects, during the study, we will ask about participants' comfort, and their participation will immediately be stopped if they are experiencing discomfort. In theory, there is a possibility that application of magnetic fields could induce a seizure. However, this has never been reported as occurring in general. In the broader field of TMS research, including riskier procedures than just motor thresholding, estimates of all seizure incidence is <0.02%, but due to its implausibility and theoretical nature, experts estimate that the likelihood of seizure during motor thresholding is much lower than this number. In the unlikely event of this occurring, trained medical professionals, including an expert in epilepsy and emergency seizure response, are on-site to respond. Furthermore, in order to mitigate this risk, we use multiple strategies. First, we screen participants based from participating in the study that present with any traits that may lower their seizure threshold or pose increased risk of seizure. These contraindications are well documented within the field and updated guidelines are released approximately every 10 years with the latest recommendation released in 2020 (Rossi et al. 2020). Contraindications screening is conducted during phone screening prior to the TMS session. Second, we have chosen our stimulation parameters to be within recommended safety guidelines. Third, we document regular trainings for personnel performing the motor thresholding procedure that matches standards recommended by expert consensus (Rossi et al. 2020, Fried et al. 2021). Fourth, we employ a comprehensive monitoring and adverse event assessment in each participant.

Rossi S, Simone Rossi, Simone Rossi, Antal A, Bestmann S, Bikson M, Brewer CC, Brockmöller J, Carpenter LL, Cincotta M, et al. Safety and recommendations for TMS use in healthy subjects and patient populations, with updates on training, ethical and regulatory issues: Expert Guidelines. Clinical Neurophysiology. 2020;132(1):269–306. doi:10.1016/j.clinph.2020.10.003

Fried PJ, Santarnecchi E, Antal A, Bartres-Faz D, Bestmann S, Carpenter LL, Celnik P, Edwards D, Farzan F, Fecteau S, et al. Training in the practice of noninvasive brain stimulation: Recommendations from an IFCN committee. Clinical Neurophysiology. 2021;132(3):819–837. doi:10.1016/j.clinph.2020.11.018

Potential Benefits:

This study has not been designed to benefit the individual participants. However, the results from this study might be used to develop future interventions using non-invasive brain stimulation.

12.2. Assessment of Safety

If the participant indicates a non-zero answer to the question about suicide on the PHQ-9, a psychiatrist or clinical psychologist will be consulted with regards to next steps.

12.3. Unanticipated Problems, Adverse Events, Serious Adverse Events

Unanticipated Problems:

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

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;

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

Adverse Event (AE) Definitions:

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

Serious Adverse Events (SAE) Definition: <insert text>

An adverse event (AE) or suspected adverse reaction is considered "serious" if, in the view of the investigator, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Grading the Severity of Adverse Events and Events of 'Special Interest':

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

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

Relatedness Definition:

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

- **Definitely Related** – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study intervention administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study intervention (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.
- **Probably Related** – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the study intervention, is unlikely to be attributed to concurrent disease or other

drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.

- **Potentially Related** – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related", as appropriate.
- **Unlikely to be related** – A clinical event, including an abnormal laboratory test result, whose temporal relationship to study intervention administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the study intervention) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).

Not Related – The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

Expectedness Definition:

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

AE and SAE Assessment, Follow-up Procedures:

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

Reporting and Documentation Procedures:

What event is reported	When is event reported	By whom is event reported	To whom is event reported
Fatal or life-threatening unexpected, suspected serious adverse reactions	Within 24 hours of initial receipt of information	Investigator	Local/internal IRB
Non-fatal, non-life-threatening unexpected, suspected serious adverse reactions	Within 48 hours of initial receipt of information	Research Personnel	Local/internal IRB

Unanticipated adverse device effects	Within 10 working days of investigator first learning of effect	Investigator	Local/internal IRB
Unanticipated Problem that is not an SAE	Within 7 days of the investigator becoming aware of the problem	Investigator	Local/internal IRB

Participant Notification of New Information:
Any new information gained during the study that may affect a participant's willingness to continue in the study will be reported to all currently enrolled participant.

12.4. Safety Monitoring

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

12.5. Study Suspension / Early Termination of the Study

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 will promptly inform research staff, study participants, and the IRB and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

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

- Determination of unexpected, significant, or unacceptable risk to participants
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable

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

13. Regulatory, Ethical, and Study Oversight Specifications

13.1. Informed Consent Process

13.1.1. Consent/Assent and Documents Provided to Participants

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Extensive discussion of risks and possible benefits of tACS will be provided to the participants.
Consent forms describing in detail the study intervention, study procedures, and risks are given to the participant and written documentation of informed consent is required before visit 1.

13.1.2. Consent Procedures and Documentation

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Consent forms will be Institutional Review Board (IRB)-approved and the participant will be asked to read and review the document. The investigator will explain the research study to the participant and answer any questions that may arise. A verbal explanation will be provided in terms suited to the participant's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. Participants must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent document will be given to the participants for their records. The informed consent process will be conducted and documented in the source document (including the date), and the form signed, before the participant undergoes any study-specific procedures. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

13.2. Study Discontinuation and Closure

See sections 7.5 and 12.5.

13.3. Confidentiality and Privacy

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

All data will only be referenced by dummy identifier code. Data will be stored on a password protected computer. A key connecting names and identifier code numbers will be kept in a locked cabinet, accessible only by research personnel. All data will be stored and analyzed on password protected computers, also only accessible by research personnel. Participants will not be identified in any report or publication about this study and there is no risk of deductive disclosure. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the IRB.

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

13.4. Future Use of Stored Specimens and Data

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

13.5. Key Roles and Study Governance

Principal Investigator	**Medical Monitor**
Flavio Frohlich, PhD	Nathan Walker MD Assistant Professor of Neurology
The University of North Carolina at Chapel Hill	UNC – Chapel Hill
Department of Psychiatry	
919-966-4584	(919) 966-9343
flavio_frohlich@med.unc.edu	nwalker@neurology.unc.edu

13.6. Safety Oversight

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

13.7. Clinical Monitoring Plan (CMP)

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

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

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

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

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

AE and SAE are clearly defined in the Master Protocol. Documents of AE and SAE can be found in the study binder on file. It is responsibility of trained research personnel to report all events to the PI.

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

13.8. Quality Assurance and Quality Control

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

13.9. Protocol Deviations

All deviations from the protocol will be addressed in study participant source documents. The researcher will complete a Protocol Deviation Log using the participant code as the identifier. This form will collect information such as the date the deviation occurred, details of what the deviation consisted of, any corrective and preventative actions that were taken as a result of the deviation, and the date that the principal investigator and IRB were notified. The principal investigator 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 study staff will be responsible for knowing and adhering to their IRB requirements.

13.10. Publication and Data Sharing Policy

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

13.11. Conflict of Interest Policy

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

14.Additional Considerations

Not applicable.

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