The Effect of Cranial Electrotherapy Stimulation on Emotional and Cellular Wellbeing in Veterans

Final Report including Study Protocol with Revisions, Statistical Analysis Plan and Results

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TABLE OF CONTENTS

PAGE
TABLE OF CONTENTS 2
LIST OF ABBREVIATIONS 4
PROTOCOL SUMMARY 6
1 KEY ROLES AND CONTACT INFORMATION 7
2 INTRODUCTION: BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE 9
2.1 Background Information 9
2.2 Rationale 9
2.3 Potential Risks and Benefits 11
2.3.1 Potential Risks 11
2.3.2 Potential Benefits 11
3 OBJECTIVES 12
3.1 Study Objectives 12
3.2 Study Outcome Measures 12
3.2.1 Behavioral Measures 12
3.2.2 Biological Measures 13
4 STUDY DESIGN 14
4.1 Basic Design Characteristics 14
4.2 Study Population 14
4.3 Study Timeframe 14
4.4 Randomization and Blinding 14
5 STUDY ENROLLMENT AND WITHDRAWAL 16
5.1 Subject Inclusion Criteria 16
5.2 Subject Exclusion Criteria 16
5.3 Strategies for Recruitment and Retention 17
5.4 Reporting Suicidal Ideation 18
5.5 Treatment Assignment Procedures 18
5.6 Subject Withdrawal 19
5.6.1 Reasons for Withdrawal 19
5.6.2 Handling of Subject Withdrawals or Subject Discontinuation of Study Intervention 19
6 STUDY INTERVENTION 20
6.1 Study Device Description 20
6.1.1 Acquisition 20
6.1.2 Packaging 20
6.1.3 Product Storage 20
6.2 Preparation and Usage of Study Device 20
6.2.1 Dosage 21
6.3 Assessment of Subject Compliance with Study Device Usage 21
6.4 Concomitant Medications/Treatments 21
7 STUDY SCHEDULE 23

7.1 Prescreening....23
7.2 Enrollment/Baseline Screening (Visit 1)....23
7.3 Intermediate Study Visit (Visit 2)....24
7.4 Final Study Visit (Visit 3)....24
8 STUDY PROCEDURES/EVALUATIONS....26
8.1 Study Procedures/Evaluations....26
8.2 Laboratory Procedures/Evaluations....26
8.2.1 Sample Draws....26
8.2.2 Pre-Visit Sample Processing....27
8.2.3 Study Visit Sample Processing....27
8.2.4 Sample Processing Directions....28
9 ASSESSMENT OF SAFETY....29
9.1 Specification of Safety Parameters....29
9.1.1 Adverse Events....29
9.1.2 Serious Adverse Events....29
9.2 Time Period and Frequency for Event Assessment and Follow-Up....29
9.3 Characteristics of an Adverse Event....30
9.3.1 Relationship to Study Intervention....30
9.3.2 Expectedness of SAEs....31
9.3.3 Severity of Event....31
9.4 Reporting Procedures....31
10 STATISTICAL ANALYSIS PLAN....32
10.1 Sample Size....32
10.2 Assessment of Primary and Secondary Outcomes....32
10.2.1 Primary Outcomes....32
10.2.2 Secondary Outcomes....32
11 ETHICS/PROTECTION OF HUMAN SUBJECTS....33
11.1 Institutional Review Board....33
11.2 Informed Consent Process....33
11.3 Subject Confidentiality....33
11.4 Future Use of Stored Specimens and Other Identifiable Data....34
12 DATA HANDLING AND RECORD KEEPING....35
12.1 Data Management Responsibilities....35
12.2 Data Collection....35
12.3 Study Records Retention....35
13 RESULTS SECTION
14 Conclusions and interpretation
13 REFERENCES....36
SUPPLEMENTAL MATERIALS....42

LIST OF ABBREVIATIONS

ACE	Adverse Childhood Experiences
AE	Adverse Event
AID	Anxiety Insomnia Depression
BD	Becton, Dickinson and Company
BOLD	Blood Oxygen Level Dependent
C	Celsius
CD-RISC	Connor-Davidson Resilience Scale
CES	Cranial Electrotherapy Stimulation
CHAPS	3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate
CHS	Center for Health Sciences
CPT	Cell Preparation Tube
CRP	C-Reactive Protein
C-SSRS	Columbia-Suicide Severity Rating Scale
CT	Cycle Threshold
DNA	Deoxyribonucleic Acid
DVD	Digital Video Disc
GLA	Greater Los Angeles
HADS	Hospital Anxiety and Depression Scale
HIPAA	Health Insurance Portability and Accountability Act
HPA	Hypothalamic Pituitary Adrenal
Hz	Hertz
IBC	Inflammatory Biology Core
ICF	Informed Consent Form
IRB	Institutional Review Board
MD	Doctor of Medicine
MHC-SF	Mental Health Continuum Short Form
MINI	Mini International Neuropsychiatric Interview
mL	Milliliter
ng	Nanogram
NP	Nurse Practitioner
OCNS	Oppenheimer Family Center for Neurobiology of Stress
OEF	Operation Enduring Freedom
OIF	Operation Iraqi Freedom

OPRS	Office of Protection of Research Subjects
PANAS-SF	Positive and Negative Affect Schedule Short Form
PBMC	Peripheral Blood Mononuclear Cells
PBS	Phosphate Buffered Saline
PCL-M	PTSD Check List - Military
PCR	Polymerase Chain Reaction
PhD	Doctor of Philosophy
PHQ	Patient Health Questionnaire
PI	Principal Investigator
PROMIS	Patient Reported Outcomes Measurement Information System
PSS	Perceived Stress Scale
PTSD	Post-Traumatic Stress Disorder
qPCR	Quantitative Polymerase Chain Reaction
R01	Research Project Grant
RN	Registered Nurse
S	Hemoglobin Single Copy Gene
SAE	Severe Adverse Event
SSL	Secure Sockets Layer
T	Telomere Gene
TPG	Telomerase Product Generated
TRAP	Telomere Repeat Amplification Protocol
UCLA	University of California, Los Angeles
VA	Veterans Affairs
µA	Microampere

PROTOCOL SUMMARY

Title:	The Effect of Cranial Electrotherapy Stimulation on Emotional and Cellular Wellbeing
Objectives:	We aim to use a CES (cranial electrotherapy stimulation) intervention to improve emotional well-being by reducing symptoms of anxiety and depression and to assess for changes in markers of cellular health - specifically, telomere length and telomerase activity
Study Design:	Randomized, double-blinded, sham controlled trial
Study Population:	18-40 year old males, with symptoms of mild to moderate anxiety and/or depression, in the absence of a psychotic disorder
Phase:	Pilot
Study Site:	UCLA Oppenheimer Family Center for Neurobiology of Stress and Resilience
Description of Intervention:	A commercially available cranial electrotherapy stimulation device that applies an electrical current to a subject's head to treat anxiety, depression or insomnia. Electrical stimulation will be applied via ear clip electrodes at the lowest setting (0.5 Hz, 100 μA) for 1 hour daily for eight weeks. In the sham group, the electrodes attached to the device will be inactive.
Study Duration:	24 months
Subject Participation Duration:	8-10 weeks
Estimated Time to Complete Enrollment:	20 weeks

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2 INTRODUCTION: BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

Chronic emotional distress can lead to detrimental biological outcomes, presumably via alterations in the hypothalamic pituitary adrenal axis (HPA axis), a sympathetic bias of the autonomic nervous system, and inflammatory dysregulation.[1] At the cellular level, impairment of the telomere/telomerase system may be a result of this dysregulation, given the descriptions of shorter telomeres (a marker of cellular aging), as well as increased markers of inflammation, in subjects with depression, anxiety and PTSD, compared to aged matched healthy populations.[2-5] These negative cellular effects of emotional distress have not been well studied. In this study we aim to use an auricular CES intervention to improve emotional well-being by reducing symptoms of anxiety and depression and to assess for changes in markers of cellular health - specifically, telomere length and telomerase activity.

This feasibility study was initially developed to study Veterans specifically, however, due to difficulty with recruitment in the Veteran population the study inclusion criteria were adjusted to include non-Veteran eligible male subjects and appropriate modifications were made in the regulatory documents.

2.2 Rationale

Auricular CES, anxiety and depression

Auricular CES benefits centrally mediated symptoms such as anxiety, depression, insomnia and pain; however, the mechanism by which the benefit occurs is not entirely clear. Similar to auricular electro-acupuncture, auricular CES is believed to act on subcortical brain structures, including the thalamus and limbic regions. This concept is supported by several small functional neuroimaging and EEG studies.[6-8] Neuroimaging during application of auricular CES shows alterations in resting brain networks, as well as a tendency toward widespread decreases in cortical activation.[9] In a study of an 8 week auricular CES intervention for pain, post treatment subjects had pain reductions as well as decreased blood oxygen dependent (BOLD) response in the basal ganglia and parahippocampal gyrus compared to subject treated with a sham device.[10]

Several small studies have shown benefits of auricular CES on negative affect symptoms. Anxiety symptoms were reduced by at least 50% in 67% of patients with generalized anxiety disorder using auricular CES in an open label study for 6 weeks.[11] Other studies have shown anxiety reductions in pre-procedure dental patients[12] and fibromyalgia patients.[13] Reduction in depression symptoms has been described in multiple reviews, with most studies only in abstract or unpublished forms, or using depression as a secondary outcome.[14-17] Pilot evaluations of electro-acupuncture and of external trigeminal nerve stimulation,

two interventions with similar targets, have also suggested that this approach may have a benefit for depression.[18,19] In one study of auricular CES using the same protocol proposed here, 115 patients with anxiety or anxiety and comorbid depression were studied over 5 weeks in a randomized, sham controlled trial, showing significant improvements in both anxiety and depression symptoms with high effect sizes (d=.8 and d=.94).[17] Due to the complexity of overlapping negative affect symptoms that lead to impaired emotional wellbeing in Veterans, we chose in this proposal to evaluate a composite measure of emotional distress (a combined anxiety and depression score) as the primary outcome. Beyond depression and anxiety, CES has been associated with reductions in insomnia and pain, both of which are also significant problems in Veterans, likely contributing to reduced emotional well-being.[14, 20-22] Data collected during program evaluation at a GLA affiliated (Veterans Integrated Service Network 22) VA Medical Center, use of CES in Veterans with comorbid PTSD, anxiety and depressive symptoms showed improvements in the Beck Depression Inventory, Beck Anxiety Index, and Insomnia Severity Index after a 1 week open label trial of 40-60 minutes of auricular CES per day (personal communication, Meredith Avedon, PsyD). While the published literature is still immature, we anticipate that CES may be a particularly useful treatment in the Veteran and non-Veteran populations given that it may target multiple symptoms causing emotional distress.

Cellular aging, anxiety, and depression

Telomeres are repeating DNA-protein complexes that cap the ends of chromosomes, protecting the DNA from damage during replication. The terminal end of the telomere shortens with successive replication as the cell ages. This aging can be accelerated in settings of stress, including symptoms of emotional distress such as anxiety and depression.[1] Reductions in telomere length have been associated with anxiety, depression, PTSD, and unhealthy lifestyles, all common conditions in OIF/OEF Veterans.[2-5] Little is known about telomere length and telomerase activity in Veterans specifically, though PTSD in Veterans has been associated with telomere shortening.[5] The mechanism through which affective symptoms and emotional stress lead to peripheral changes in the telomere/telomerase system has not been completely elucidated but is likely the result of a dysregulated hypothalamic pituitary adrenal axis, impaired anti-inflammatory pathways, and a sympathetic shift of the autonomic nervous system, all leading to greater cellular stress. It is unclear whether reductions in telomere length create a permanent "biological scar" during periods of systemic stress or whether compensatory increases in telomerase can lengthen the telomeres, as there is contradictory evidence for both situations in human studies.[1] Mind-body interventions, such as meditation, as well as pharmacological approaches, have been shown to improve telomerase levels and telomere length.[1,23]

In this study we hypothesize that the complex chronic stressors, which include anxiety, depression, PTSD, insomnia, and chronic pain will lead to biological stress at the cellular level. This stress will have resulted in greater than normal telomere shortening and suppressed telomerase, as has been described in PTSD.[24] Improvement in key symptoms of emotional distress, anxiety and depression, is expected to lead to decreased biological stress, and increases in telomerase activity, possibly with improvements in telomere length, though the latter may require a longer follow up.

2.3 Potential Risks and Benefits

The study devices used are commercially available and have an excellent risk profile based on previous human clinical studies and post marketing data.

2.3.1 Potential Risks

The most common side effects of the device are dizziness, headache, and local skin irritation. Dizziness and headache most commonly occur at higher current levels but will be minimized by having the first electrical stimulation session performed at the research center to make sure the study protocol is tolerated. Subjects who cannot tolerate the study protocol will be dropped from the study. Phlebotomy has a low risk of bruising, swelling or pain at the site, and a very low risk of infection.

The collection of data contains the inherent risk of loss of privacy. This risk will be minimized by adequate training of all study staff according to institutional guidelines, use of unique subject identifiers, and by keeping data in password protected computer databases and/or locked cabinets. An informed consent document was approved by the local Institutional Review Boards at UCLA and a recruitment waiver was obtained from the IRB at the Greater Los Angeles VA. All subjects will read and document understanding of the consent form prior to enrolling in the study.

2.3.2 Potential Benefits

This study may improve emotional well-being for individual subjects with proper and consecutive use of the CES device but any beneficial effects are not certain to occur. It is hoped that the research will also benefit Veterans and individuals suffering with anxiety and/or depression by increasing awareness of these conditions and further research to identify improved diagnostic markers for anxiety and depression. Societal benefits of this study include the potential addition of a non-invasive, non-medication treatment options for patients with anxiety and/or depression. Cellular marker identification and an improved understanding of the physiological mechanisms behind CES therapies may enhance and improve therapies currently available.

3 OBJECTIVES

3.1 Study Objectives

We anticipate significant improvements in emotional well-being associated with eight-weeks of daily CES treatment. Scores on the individual and combined anxiety and depression subscales of the HADS will decrease. Improvements in other aspects of mental health (measured with the MHC-SF) are expected as a secondary effect of the improved negative affect measured in the HADS. An increase in telomerase is expected at 8 weeks. In addition we expect improvements in the PROMIS measures of fatigue, sleep disturbance, and pain interference.

Biological samples will be retained for measurement of additional inflammatory gene regulation pathways in peripheral immune cells if additional funding can be obtained for processing and analysis of the samples. We anticipate that inflammatory gene profiles would be improved in subjects who have improved emotional wellbeing with CES treatment.

3.2 Study Outcome Measures

3.2.1 Behavioral Measures

1. PRIMARY OUTCOME: The Hospital Anxiety and Depression Scale (HADS) will be used to measure emotional distress/well-being. The score for each of the two components will be summed to yield a composite score. The severity of emotional distress will be defined as normal range (0-7), mild (8-10), moderate (11-14) or severe (15-20).[25] Pre minus post intervention score will be assessed.

2. EXPLORATORY OUTCOME: The Mental Health Continuum Short Form (MHC-SF), which measures psychological and social well-being, will be used to assess improvements in two forms of human flourishing or happiness, hedonic (self-gratification) and Eudaimonic (having a noble purpose) well-being.[27]

3. EXPLORATORY OUTCOME: PROMIS-SF measures will be collected to assess ancillary symptoms in the domains of: fatigue, sleep disturbance, sleep related impairment, and pain interference.

4. EXPLORATORY OUTCOME: The PCL-M is a frequently used instrument to screen for PTSD in the military population, correlates well with a clinical interview and has been shown to be responsive to symptom change.[24]

3.2.2 Biological Measures

The primary biological outcome will be telomerase activity determine using the telomere repeat amplification protocol (TRAP), performed as previously described with minor modification.[28,29] Isolated PBMC are suspended in Chaps lysis buffer and telomerase extract obtained. Extracts are incubated to build telomere repeats, resulting in the product generated due to telomerase activity. To visualize, the PCR reaction included a labeled primer to amplify signal. The PCR product is then run in an acrylamide gel and detected by fluorescent imaging. Calculation of telomerase product generated (TPG) per 10,000 PBMC is performed using ImageQuant software, which takes the fluorescence intensity within a specified parameter and yields a numerical value representing TPG per 10,000 cells. Telomere length will be determined using real time quantitative polymerase chain reaction (qPCR) methodology as described previously with minor modifications.[30,31] Peripheral blood mononuclear cells (PBMC) are isolated and genomic DNA extracted. Using the standard curve method, cycle threshold (CT) values are plotted on a standard curve of human genomic DNA to estimate an ng/microliter concentration value. Telomere length values are expressed as the ratio of the estimated concentration generated by PCR of the telomere gene (T) divided by the hemoglobin single (S) copy gene = (T/S).

4 STUDY DESIGN

4.1 Basic Design Characteristics

This is a pilot, randomized, double-blinded, sham controlled study on the effect of transcutaneous electrical nerve stimulation on emotional and cellular wellbeing. This is a single center study and all visits will occur at OCNS.

Subjects will be asked to use the CES device daily for 8 weeks. They will be provided with a tracking diary to record the start and stop time of usage daily. Device must be used at approximately the same time of day. Failure to comply is a violation of study protocol and will result in subject withdrawal (see 5.6.1).

4.2 Study Population

Approximately 55 male subjects will be screened in an effort to obtain 22 subjects in each treatment group after randomization. Due to the difficulty with recruitment of eligible Veterans in this feasibility pilot, the study period was extended and non-Veterans meeting the other inclusion criteria were recruited. The study enrollment was limited to 24 completed subjects based on insufficient resources to complete the study beyond the extended recruitment period.

4.3 Study Timeframe

The study timeframe consists of a screening period of 1-2 weeks (commencing between Week -2 to Week -1 and ending on Week 1; see 7.1 and 7.2) followed by an 8-week treatment period (Week 1-Week 8). A flowchart showing the study schedule is provided in **Error! Reference source not found.**.

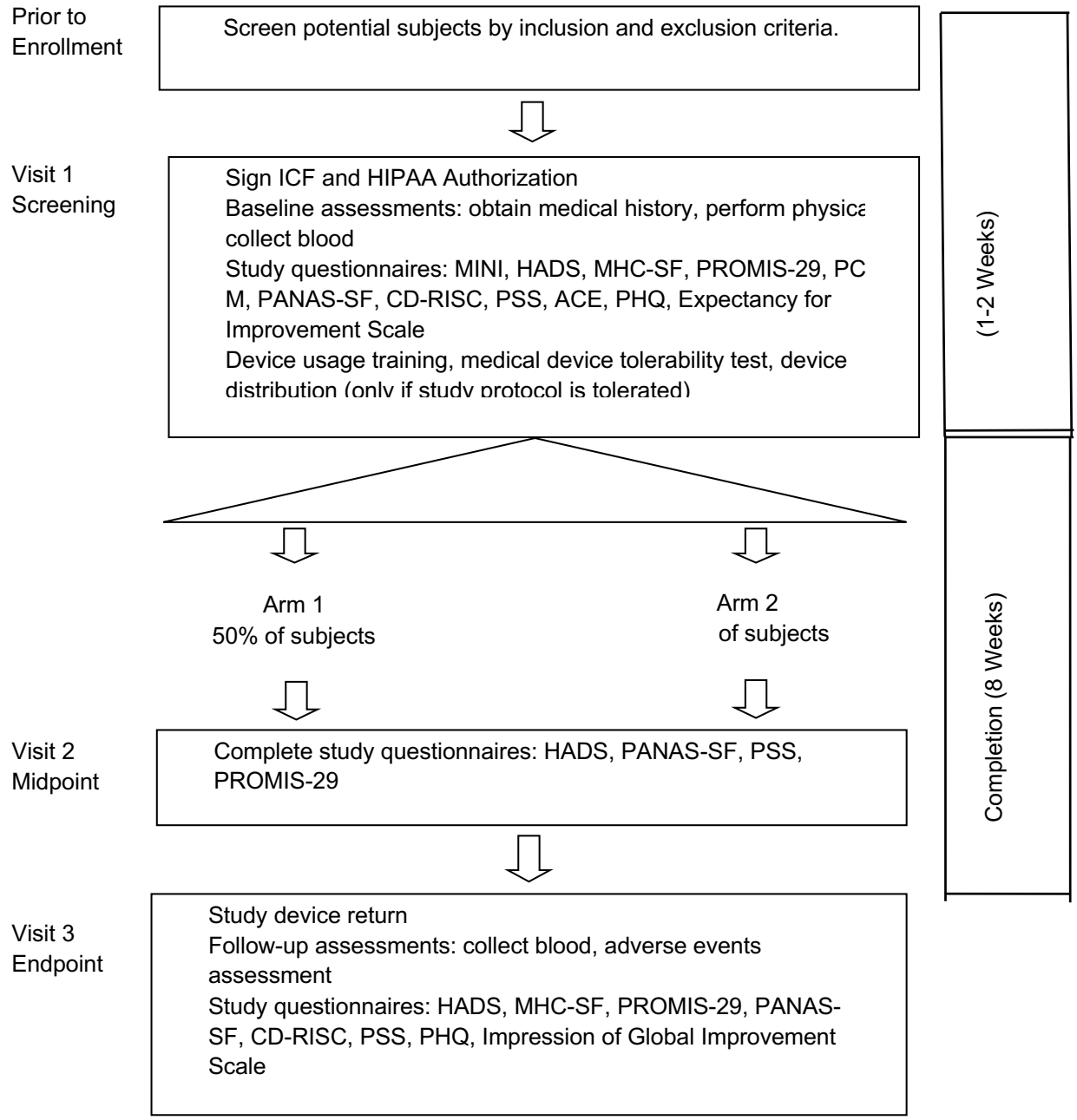
The expected duration of subject participation is about 8-10 weeks..

4.4 Randomization and Blinding

This is a randomized, double-blinded, sham controlled study. The active and sham devices are identical in appearance and will only differ by the labeling of a unique serial number. In the sham devices, the electrodes attached to the device will be inactive. The subjects will be instructed that the device is set to a low level so that the current is not detectable but still effective. The current will not be detectable in both active and sham devices in order for adequate blinding to occur.

To ensure that the allocation of subjects to treatment is properly concealed, the PI and research personnel will not know which devices are active or sham. The sponsor will provide the code to determine which devices are active and which are sham. Code will be in a sealed envelope that will not be opened until the end of data collection.

Schematic of Study Design



5 STUDY ENROLLMENT AND WITHDRAWAL

55 Veterans with symptoms of mild to moderate anxiety and depression, in the absence of a psychotic disorder, will be recruited in order to complete 22 subjects per treatment group based on an estimated 25% rate of subject attrition.

5.1 Subject Inclusion Criteria

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

1. Male, Veteran or non-Veteran
2. Within the age range of 18-40 years old
3. On a stable regimen for anxiety, depression, psychiatric or mental health treatment (pharmacological or non-pharmacological) for the past 3 months
4. No active suicidal ideation or psychosis (including schizophrenia and bipolar disorder)
5. No uncontrolled or progressive severe medical illness (e.g., cancer, uncontrolled diabetes mellitus, active cardiac disease)
6. No use of a pacemaker or any other implanted electrical device
7. No alcohol consumption greater than 2 units daily
8. No daily use of illicit drugs or marijuana
9. Ability to independently complete the in-person study questionnaires and sign ICF without assistance
10. Willing to comply with all study procedures and be available for the duration of the study
11. No participation in another clinical trial study

5.2 Subject Exclusion Criteria

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

1. Not male
2. Younger than 18 years old or older than 40 years old
3. Not on a stable regimen for anxiety, depression, psychiatric or mental health treatment (pharmacological or non-pharmacological) for the past 3 months

4. Active suicidal ideation or psychosis (including schizophrenia and bipolar disorder)
5. History of inpatient treatment or suicidal ideation within the last year
6. Use of a pacemaker or any other implanted electrical device
7. Current alcohol and/or substance abuse of illicit drugs or marijuana
8. Unable to independently complete the in-person study questionnaires and sign ICF due to impaired cognitive function
9. Unwilling to comply with all study procedures
10. Unavailable for the duration of the study
11. Current participation in another clinical trial study
12. Any other condition that the investigator believes would jeopardize the safety or rights of the subject or would render the subject unable to comply with the study protocol or make use of acquired data non-analyzable

5.3 Strategies for Recruitment and Retention

Study participants will be recruited with flyers on UCLA and VA campuses, Internet web advertisements posted on non-interactive Facebook pages and Craigs List, and through direct contact from GLA VA physicians. Most subjects are expected to be recruited from the VA Physical Medicine and Rehabilitation clinic and from other primary providers in ancillary clinics at the VA. Physicians in these clinics are aware of this research study and will give information about who to contact if a Veteran is interested. Physicians will refer the potential study participants to the study team.

Subjects will be compensated for study participation as follows:

$50.00 for completing screening visit 1, $50.00 for completing the mid-study visit 2 and questionnaire completion, and $100.00 for the final visit and completing the final set of questionnaires.

Only subjects who complete a minimum of 5 of 7 days per week compliance will be eligible to complete the final visit and thus be eligible for that payment. Subjects enrolled in study will be contacted weekly to assure subject compliance.

Parking will be provided at all visits.

The stipend payment subjects will receive will be up to $200.00. If the subject withdraws from the study before finishing, that person will be paid for the study visits completed according to the schedule above.

5.4 Reporting Suicidal Ideation

To minimize risk, patients will be selected based on mild to moderate but not severe depression and/or anxiety, and who are on a stable therapeutic regimen (medication, psychotherapy, or stable without treatment) to reduce the risk of harm with a new intervention. Patients will be interviewed for both physical and psychiatric symptoms. Patients with a history of suicide attempt in the past year, who express having an intention of acting on suicidal thoughts as measured by a positive answer to the Columbia-Suicide Severity rating scale questions 4-6, or have other clinical risks on interview deemed by the investigator to be exclusionary will be excluded from the study and referred to a mental health professional, primary care provider, or emergency room after assessment of the patient. If the patient already has a mental health care or primary care provider, the provider will be contacted by the medical staff, with the patient's permission, to update them on the subjects's change in condition. All subjects will be asked at entry to the study to sign a HIPPA release and provide their clinicians contact information, so that this option is available in the event of a clinical emergency. The Veteran's Crisis line number will be provided to all Veteran subjects (1-800-273-8255). Subjects whose answers to the Columbia screener "since last visit" questionnaire show an increase in suicidal thoughts will be discharged from the study after determination of appropriate follow up as described above. No attempt to control psychiatric medication will be made in this study. Types and doses of medications taken by the subject will be carefully documented and examined in post hoc analyses.

5.5 Treatment Assignment Procedures

Stratified random sampling will be performed using SAS Proc Plan to generating random sequences and dispensing one of the Alpha-Stim AID kits provided by the sponsor (50% of kits active, 50% sham) and coded with unique serial numbers.

5.6 Subject Withdrawal

Study participation is voluntary and subjects have the right withdraw from the study at any time. The investigator may terminate a subject's participation if necessary.

5.6.1 Reasons for Withdrawal

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

An investigator may terminate a study subject's participation in the study if:

- Any clinical AE or SAE, laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the subject.
- The subject meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation.
- Non-compliance with study protocol

5.6.2 Handling of Subject Withdrawals or Subject Discontinuation of Study Intervention

An adverse events report will be collected from subjects who choose to withdraw from the study. Subjects will be required to return study device to research personnel.

In the event of an (S)AE, every effort will be made to obtain follow-up data on any subject who discontinued device treatment due to an (S)AE. To the extent possible, any withdrawn subject will be followed until the (S)AE has resolved.

6 STUDY INTERVENTION

6.1 Study Device Description

The study device is a commercially available cranial electrotherapy stimulation device that applies an electrical current to a subject's head to treat anxiety, depression or insomnia.

6.1.1 Acquisition

Study device will be supplied and shipped by sponsor after IRB approval.

6.1.2 Packaging

Study device comes complete with the following accessories:

- One set of Earclip Electrodes
- 256 Earclip Electrode Pads
- 15ml Alpha Conducting Solution
- Two AAA batteries
- Storage case
- AID DVD
- Neck lanyard
- Illustrated Owner's Manual (multi-lingual Owner's Manual also available)

Weight: 2.0 lbs

Dimensions: 9 x 8 x 2 in

6.1.3 Product Storage

Study device should be stored inside provided storage case. The device and additional provided accessories should be kept in a safe place for subject use only and away from water.

6.2 Preparation and Usage of Study Device

Study product: Alpha-Stim AID

- Place earclip electrode pads to earclip electrodes
- Attach earclip electrodes to CES device
- Wet each electrode with Alpha Conducting Solution

- Clip electrodes to ears
- Set device frequency to 0.5 Hz and 100 μA
- Set current to maximum of 600 microamperes, or to a comfortable level on the head
- Set probe timer at 60 minutes
- Remove earclip electrodes from ears after 60 minutes
- Unplug earclip electrodes from device and dispose of electrode pads
- Store device and additional accessories in storage case

6.2.1 Dosage

The dose selection (device frequency of 0.5 Hz and 100 μA) is based on previous positive studies of the device for anxiety, depression, and insomnia. The dose selected is not detectable by the subject, so that adequate blinding can occur.

6.3 Assessment of Subject Compliance with Study Device Usage

Subjects will be provided with a tracking diary to record the start and stop time of daily usage, duration of usage, and any side effects if applicable. The research assistant will call every day during the first week of device treatment to ensure subjects are using the device, following instructions, and recording device usage times. Any reported side effects, additions or changes to medications or health care regime (i.e. non-pharmacological treatment), and adverse events will be noted. Subjects who note worsening of their anxiety or depression will be called by the study PI and if needed, they will be referred to their primary care of mental health provider for evaluation as appropriate, or if necessary for emergency treatment. During weeks 2-8 of device treatment, the research assistant will call once a week instead of daily.

6.4 Concomitant Medications/Treatments

Subjects who are treated for anxiety, depression, psychiatric or mental health must be on a stable regimen for the past 3 months. A concomitant medication report will be collected during each visit at OCNS.

Patients who add or change their medications or health therapy will be identified via weekly telephone check-up. We will allow necessary additions or changes in medications, specifically psychiatric medications, from the subject's personal provider except for the use of narcotics/opiates. Subjects who use narcotics/opiates after study enrollment will be withdrawn by PI upon PI notification.

Any subjects who express suicidal ideation will be referred to and followed up with their own primary care provider or psychiatric MD if they are able to contract for safety or to a psychiatric emergency department if they indicate a great risk of harm to themselves. In the absence of these, they will be referred to and followed up at a clinic/country medical system, which can provide proper care. All subjects sign a HIPAA or Release of Medical records if we feel this is a concern, enabling us to request past medical/psychiatric records if necessary.

No prohibitions will be made for changing health therapy but we will record and account for any changes in our analysis. We assume such changes in therapy will be uncommon but if they occur they will be equally balanced between the randomized groups.

While participating in this research study, subjects are not allowed to take part in any other research project. Simultaneous medications or treatments may invalidate the results of this research, as well as that of the other studies.

Changes in concomitant medication and any adverse events (see 9.1.1) are assessed at each visit, documented in subject's file, and reported to the PI for assessment.

7 STUDY SCHEDULE

7.1 Prescreening

Potential subjects who respond to the study advertisement will undergo a brief (5 to 10 minute) phone screening by research assistant to assess potential eligibility prior to Visit 1. The prescreening will require potential subjects to respond verbally to approximately 10 yes/no medical history questions in order to determine whether a screening visit should be scheduled.

Subjects who seem eligible and available for the duration of the study will be scheduled for a screening visit at OCNS.

7.2 Enrollment/Baseline Screening (Visit 1)

This visit will occur at the Oppenheimer Family Center for Neurobiology of Stress. It will take about 3 hours to complete the screening and device training process. The research team will verify OEF/OIF Veteran status (i.e. DD 214) before study enrollment if the subject identifies as a Veteran. The potential subjects will first review and sign ICF and HIPAA Authorization with either the RN, FNP, or MD. All are certified to obtain informed consent (nursing or physician license). Subjects will be identified by the PI, Dr. Tillisch, as possibly eligible after the clinic visit and the study questionnaires have been completed and scored. Medical history will be reviewed and a physical will be performed as well. The assessment questionnaires include:

- A standardized psychiatric evaluation (MINI)
- Hospital Anxiety and Depression Scale (HADS)
- Mental Health Continuum Short Form (MHC-SF)
- Patient Reported Outcomes Measurement Information System (PROMIS-29)
- Positive and Negative Affect Schedule Short Form (PANAS-SF)
- Connor-Davidson Resilience Scale (CD-RISC)
- Perceived Stress Scale (PSS)
- Patient Health Questionnaire (PHQ)

- Columbia-Suicide Severity Rating Scale (C-SSRS) Self Report Screener - Recent

We will use the HADS to assess symptom severity defined as normal range (0-7), mild (8-10), moderate (11-14) or severe (15-20). Subjects with impaired emotional wellbeing as defined as a minimum combined HADS score of 14 will be included. Subjects who score >15 will be excluded in the study and will be given referrals to their personal provider or community mental health resources. (See “Reporting Suicidal Ideation” Section 5.4)

Subjects who express interest in participating and meet the inclusion criteria will be trained to use the CES device. They will be instructed that the device is set to a low level that should not be detectable. Subjects will have their first 1 hour treatment at OCNS (commencing Week 1 of the study). Subjects who tolerate the device treatment will then have blood drawn for baseline biological measures. They will be informed that this is a randomized controlled trial and they may or may not have an active device kit. Subjects will be sent home with a device, instructions for daily treatment, and a patient diary to track usage and side effects if applicable.

7.3 Intermediate Study Visit (Visit 2)

This visit will occur at the Oppenheimer Family Center for Neurobiology of Stress. This visit will take approximately 1 hour and will occur Week 4 ± 1 week. Subjects must complete this set of study questionnaires:

- Hospital Anxiety and Depression Scale (HADS)
- Positive and Negative Affect Schedule Short Form (PANAS-SF)
- Perceived Stress Scale (PSS)
- Columbia-Suicide Severity Rating Scale (C-SSRS) Self Report Screener - Since Last Contact
- Patient Reported Outcomes Measurement Information System (PROMIS-29)

Subjects will have vital signs and weight measured by a nurse. Adverse events and concomitant medication report will be collected as well. The study coordinator will collect the tracking diary for weeks 1-4 and provide another tracking diary to complete for weeks 5-8.

7.4 Final Study Visit (Visit 3)

This visit will occur at the Oppenheimer Family Center for Neurobiology of Stress. This visit will take approximately 2 hours and will occur after 8 weeks of

treatment, Week 8 + 1 week. Subjects will be asked to bring the CES device to return. The study coordinator will collect the tracking diary for weeks 5-8. The subjects must complete the final set of questionnaires:

- Mini International Neuropsychiatric Interview (MINI)
- Hospital Anxiety and Depression Scale (HADS)
- Mental Health Continuum Short Form (MHC-SF)
- Patient Reported Outcomes Measurement Information System (PROMIS-29)
- Positive and Negative Affect Schedule Short Form (PANAS-SF)
- Connor-Davidson Resilience Scale (CD-RISC)
- Perceived Stress Scale (PSS)
- Patient Health Questionnaire (PHQ)
- Columbia-Suicide Severity Rating Scale (C-SSRS) Self Report Screener - Since Last Contact
- Impression of Global Improvement Scale

The subjects will have vital signs and weight measured and have the final blood draw performed by a nurse. Adverse events and concomitant medication report will be collected as well.

8 STUDY PROCEDURES/EVALUATIONS

8.1 Study Procedures/Evaluations

Electronic assessment questionnaire data will be collected from subjects during the visits at UCLA OCNS using SurveyMonkey (www.surveymonkey.com) and unique subject identifiers. Survey Monkey states they utilize some of the most advanced technology for Internet security commercially available today. Unique usernames and passwords are required and must be entered each time a user logs on. When a user accesses secured areas of our site, Secure Sockets Layer (SSL) technology protects user information using both server authentication and data encryption, ensuring that user data is safe, secure, and available only to authorized persons. Servers are kept in a locked cage. Digital surveillance equipment monitors the data center. A firewall restricts access to all ports except 80 (http) and 443 (https). Intrusion detection systems and other systems detect and prevent interference or access from outside intruders. QualysGuard network security audits are performed weekly. McAfee SECURE scans performed daily.

The subjects will complete the aforementioned study questionnaires above (see "Study Schedule" Section 7) and the assessments will be scored per published guidelines.

All paper source data collected at UCLA will only contain unique subject ID numbers. Research staff will know matching of ID with subject for data collection purposes but this will not be written on documents in the field. The documents will be coded before being stored in subject's file. Files will be secured in a locked cabinet in a locked room.

8.2 Laboratory Procedures/Evaluations

8.2.1 Sample Draws

Sample blood draws will be done by either the nurse coordinator or nurse practitioner. 8 mL BD Vacutainer® CPT™ Cell Preparation Tubes with Sodium Citrate will be used to store the blood draws. 8 mL of blood will be drawn during visit 1 and visit 3, so 16 mL of blood total will be drawn from each subject.

The blood draws will be performed at the OCNS clinic, CHS 42-128, 10833 LeConte Ave Los Angeles, CA 90025 and transferred immediately to the Inflammatory Biology Core (IBC) Laboratory, 300 Medical Plaza, Room 3160, for processing and storage.

8.2.2 Pre-Visit Sample Processing

- Patient must be scheduled at least one week in advance by notifying through e-mail Stephanie, Nancy, Dr. Breen with the following: patient ID, date, time point of visit (pre- or post-op), and estimated time of delivery of blood samples.
- If the new or rescheduled visit is less than one week away, please call Stephanie first at the IBC Lab phone (310-825-0302 or ext 50302) to confirm the ability of the lab to handle the specimens. When confirmed, then please email Stephanie, Nancy, Dr. Breen
- Prepare tubes, labels (CES ID#, time point, and date), transport containers in advance

8.2.3 Study Visit Sample Processing

1. Have the following tubes ready to be drawn in the following order:
    i. 1 CPT tube, 8 mL (pick up as needed from IBC Lab)
2. Complete top portion of "CES Requisition Form" for IBC Lab
3. Have labels with participant's study ID # and date ready
    i. 1 x 8 mL CPT tube handling

        <u>BE SURE TO FILL CPT TUBE COMPLETELY</u>.

        Once the CPT tube is filled, **remove from blood draw apparatus and immediately but gently invert tube 8 to 10 times** to mix anticoagulant additive with blood. DO NOT SHAKE. Vigorous mixing can cause hemolysis. Place mixed tube upright in rack at room temperature in transport container; insure that container is closed to shield tube from light.

        NOTE: Since this BD Vacutainer® CPT™ Tube contains chemical additives, it is important to prevent possible backflow from the tube with its attendant possibility of adverse reactions to the patient. To guard against backflow, the following precautions should be taken when drawing blood into the tube:

        - Keep patient's arm in the downward position during the collection procedure
        - Hold the tube with the stopper uppermost
        - Make sure the tube contents do not touch the stopper or the end of the needle during collection procedure

4. **Deliver *ASAP to IBC lab by 4 p.m. at the latest***, 300 Med Plaza, third floor, Room 3160 (main Lab):

   - **Room temperature transport container**
     - Place transport container containing rack with CPT and requisition form on black crate just inside door

   If no lab staff is present in room 3160 where samples are dropped off, please check in room 3152 (opposite Deb Garet's office) to let someone know samples have arrived.

   (If delayed after 4 p.m. and IBC Lab is closed, lavender top tubes may be held in refrigerator overnight and delivered to IBC Lab the next day; CPTs should be discarded)

   Blood delivered to IBC Lab will be processed and stored for CPTs = PBMC (pellets for future Telomere and Telomerase)

8.2.4 Sample Processing Directions

Upon receipt of CPT tubes upright in rack at RT:

1. Within two hours of blood draw, begin isolation of PBMC utilizing CPT to PBMC protocol—***be sure to re-mix CPT tube by inverting 8 times before centrifugation***.
2. Count cells record number of PBMC's on requisition.
3. Proceed according to the following algorithm:
   a. $2x10^6$ cells for pellet in PBS for DNA extraction.
   b. $1x10^6$ cells for telomerase extraction in CHAPS buffer.
4. Freeze PBMC for DNA pellet(s) at -80°C to store for future extraction.
5. Perform telomerase extraction on fresh cells per IBC protocol.

9 ASSESSMENT OF SAFETY

9.1 Specification of Safety Parameters

Safety assessments will include recording of AEs, which will be followed until the event has resolved or the condition has been deemed stable by the Investigator. At each visit, the Investigator will evaluate the subject to determine whether any AE has occurred. AEs may be directly observed, spontaneously reported by the subject, or obtained by questioning the subject at each study visit. Subjects should be questioned in a general way without asking about the occurrence of specific symptoms; at each visit, they will be asked standard questions to elicit any medically related changes in their well-being.

All SAEs will be reported to regulatory authorities and the site IRB within required time frames.

9.1.1 Adverse Events

The Investigator is responsible for reporting all AEs observed or reported during the study, regardless of clinical significance or potential causal relationship to study medication.

An AE is any untoward medical occurrence in a human subject administered the study device, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research. Subjects will be instructed to contact the Investigator at any time after randomization if any symptoms develop.

9.1.2 Serious Adverse Events

An SAE is defined as any event that results in death, is immediately life-threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity or is a congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered SAEs when, based on appropriate medical judgment, they may jeopardize the subject and require medical or surgical intervention to prevent one of the outcomes listed in this definition.

9.2 Time Period and Frequency for Event Assessment and Follow-Up

All AEs reported by subjects or observed during the study will be recorded. All AEs shall be recorded in subject's file regardless of perceived causality. AEs will be assessed from the first device treatment (screening visit) to the last device treatment. AE form will be collected during each study visit at OCNS.

9.3 Characteristics of an Adverse Event

9.3.1 Relationship to Study Intervention

The Investigator is obligated to estimate the relationship between the device and the occurrence of each AE or SAE by using his or her best clinical judgment. Other elements, such as the history of the underlying disease, concomitant therapy, other risk factors, and the temporal relationship of the event to the investigational product, will be considered and investigated.

To assess relationship of an event to study intervention, the following guidelines are presented in Table 1.

Table 1 Assessment of Causality/Relatedness of AEs and SAEs

Categories	Definition
Definitely related	This relationship suggests that a definite causal relationship exists between the device treatment and the AE, and other conditions (concurrent illness, progression/expression of disease state, or concurrent medication reaction) do not appear to explain the event.
Probably related	This relationship suggests that a reasonable temporal sequence of the event with device treatment exists and, based upon the known side effects of device treatment, known or previously reported adverse reactions to device treatment, or judgment based on the Investigator's clinical experience, the association of the event with the study device seems likely.
Possibly related	This relationship suggests that device treatment may have caused or contributed to the AE (i.e., the event follows a reasonable temporal sequence from the time of device treatment and/or follows a known response pattern to the study treatment but could also have been produced by other factors).
Not related	This relationship suggests that there is no association between the study device and the reported event.

9.3.2 Expectedness of SAEs

The Study PI will be responsible for determining whether an SAE is expected or unexpected. An adverse event will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the intervention.

9.3.3 Severity of Event

The Investigator will make an assessment of severity for each AE and SAE reported during the study. The assessment will be based on the Investigator's clinical judgment. The severity of each AE should be assigned to one of the categories shown in Table 2.

Table 2 Common Adverse Event Grading Scale

Grade	Definition
Mild	Aware of sign or symptom, but easily tolerated
Moderate	Discomfort enough to cause interference with usual activities
Severe	Incapacitating, with inability to work or perform usual activities

An AE that is assessed as severe should not be confused with a SAE. Severity is a category used for rating the intensity of an event (such as mild, moderate, or severe), and both AE(s) and SAE(s) can be assessed as mild, moderate, or severe. An event is defined as serious when it meets one of the predefined outcomes described in 9.1.2.

9.4 Reporting Procedures

AE's that meet the criteria for reporting per the UCLA IRB guidelines are reported to the IRB within 10 working days of awareness where the UCLA IRB is the responsible IRB, and is determined by the UCLA PI to be either:

- Unexpected, and related or possibly related to the research activity, and serious (i.e. placing subjects art greater risk of harm than previously known)

  or

- Expected and related to but indicating a higher severity or frequency of occurrence (i.e. a trend) than was known or described in the current approved ICF and therefore anew identified risk.

SAEs or deaths that are unanticipated, serious, and possibly related to the study intervention will be reported to the UCLA IRB in accordance with their requirements which is within 3 working days of awareness.

10 STATISTICAL ANALYSIS PLAN

10.1 Sample Size

G*Power 3.1 was employed to calculate the number of subjects needed per group to detect a large group differences, slightly less than a 1 standard deviation difference (Cohen's effect, size, d=.80) in the effect of daily CES treatment on emotional well-being and PBMC telomerase activity. Based on a one tailed superiority test of improvement (active > sham CES treatment) and an alpha = .05, 22 subjects per group are required to provide adequate statistical power (80%) for the analysis (Noncentrality parameter=2.59, Critical t (40)=1.68. To meet this criteria we will include 22 completed subjects per group.

10.2 Assessment of Primary and Secondary Outcomes

Intention to treat and completed group analyses will be performed for the behavioral measures. Because the utility of CES in effecting telomerase, telomere length, or CRP has not been established, the biological measures will be assessed only for those subjects who have both pre and post treatment data points.

10.2.1 Primary Outcomes

One tailed t-tests for independent groups will be performed to test the hypothesis that active CES device will be superior to sham in decreasing the combined HAD score. This will be accomplished by Contrast analysis within the framework of a random effects general linear mixed effects (RE GLMM) model.

10.2.2 Secondary Outcome

One tailed t-tests for independent groups will be performed to test the hypothesis that participants using the active vs sham CES device will have smaller reductions in telomere length and higher telomerase levels at post compared to pre-treatment.

10.2.3 Exploratory Outcomes

- One tailed t-tests of independent groups will be performed to test the hypotheses that active CES device will be superior to sham in improvement of PROMIS domains, MHC-SF, CD-RISC, PSS, PHQPANAS-SF and impression of global improvement.

11 ETHICS/PROTECTION OF HUMAN SUBJECTS

11.1 Institutional Review Board

The protocol, Informed Consent Forms, recruitment materials, and all subject materials will be submitted to the IRB for review and approval. Approval of both the protocol and the Informed Consent Form must be obtained before any subject is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented in the study.

11.2 Informed Consent Process

Informed consent is a process that is initiated prior to the individual agreeing to participate in the study and continues throughout study participation. Before coming to the clinic, the study is explained via phone screening using the IRB-approved prescreening script. All interested volunteers are offered a copy of the consent form beforehand in order to review and ponder the consent by themselves or with family, friends or advisors before their screening date. At every stage of the process, they will be given opportunity to ask questions or voice concerns that they may have.

The informed consent and study documents will be in English language only therefore only persons fluent in reading English will be eligible to enroll into the study. The consent is written to ensure comprehension at a 6th-8th grade level. No vulnerable persons, as defined by the Office of Protection of Research Subjects (OPRS/IRB), will be targeted. The PI and research personnel (co-investigator, RNs, research assistant) will be responsible for inviting subjects to participate. The study will be explained in detail and they will be allowed as much time as needed to read, comprehend and ask any pertaining questions. After the subject has read the consent form at the UCLA OCNS private clinic exam room area, the investigator will review the study and answer any questions or concerns regarding participation. Subjects will be asked to summarize the information provided to ensure comprehension. It will be explained that they are allowed to withdraw consent at any time without retribution. The consent process will be documented in the research record.

11.3 Subject Confidentiality

Subject's names and study information will be kept on a password protected database, and will be linked only with a unique 4-digit study identification number for this research. The only people who will have access to the data or the codes will be research team associated with this study. All other data is stripped of personal identifiers and stored in an encrypted Microsoft Access database. All electronic data in the computer database is password protected. Paper source documents are stored in a locked office with locked cabinets in a secured office location. Only authorized research personnel will have access to the locked office and cabinets.

11.4 Future Use of Stored Specimens and Other Identifiable Data

These procedures will be taken to protect the confidentiality of the subjects who donate samples. The procedures by which samples will be stored including the information that will be attached to samples. The process by which the donor's identity will be protected (process of coding and de-identifying samples) including the location where the information that links a sample to the identity of the donor is kept and who has access to this information.

Although the samples containing genetic material will be coded and no subject identifiers will be attached to the samples, the codes will be maintained by the PI or the research assistant in the records along with the date of collection on the tube containing genetic material and in the specimen log book. This will allow protecting the identity of the donor from anybody who has access to the storage, but is not working on the study. The Samples will be coded but not anonymous. Therefore the policy will read:

Policy: Samples will be coded to protect the identity of the donor from anybody who is not a part of the research team. The link will be maintained between subject identifiers and stored samples in the PI's records. Samples will be stored in a manner that optimizes the viability of the specimen and the utility of the cells for research. All equipment used in preparation or storage of human specimens shall be subject to routine maintenance to ensure optimal preservation of specimens.

Process: The participant will specify within the consent form whether they would like to the samples stored for future research. On receipt of a sample the PI or study coordinator will assign a code to the sample and record the code and the date of collection on the tube containing genetic material and in the specimen logbook. This will be the only link between the samples and the subjects accessible only to the PI and members of the research team. The sample will then be processed in accordance with the research needs of the study. The samples will not be shared.

Sharing: The participant sample will not be shared with other researchers outside the research team. Since the sample will only be shared within the research team, the only set process for sharing (within the team) will be a written request to the PI. Only after approval by the PI, will the samples be release to the co-investigators. Only the subjects ID will accompany the sample.

Processing of sample upon leaving UCLA: In the event that the PI leaves UCLA, then the samples will be destroyed.

12 DATA HANDLING AND RECORD KEEPING

12.1 Data Management Responsibilities

Data collection and accurate documentation are the responsibility of the study staff under the supervision of the investigator. All source documents and laboratory reports must be reviewed by the study team and data entry staff, who will ensure that they are accurate and complete. Unanticipated problems and adverse events must be reviewed by the investigator.

12.2 Data Collection

Paper source documents with data collected at UCLA will be coded and kept in locked cabinets. A coding key and any collected identifiers (e.g., phone, address) will be kept in a separate locked cabinet by the PI. All data will be coded upon storing and before data analysis.

Electronic data collected at UCLA will be done using SurveyMonkey and will be coded. All data will be stripped of personal identifiers and stored in an encrypted Microsoft Access database. All data in the computer database will be password protected. Only authorized research personnel will have access to the password protected computer database.

12.3 Study Records Retention

Upon completion of the study, the coding key and all identifiers will be destroyed, rendering the data anonymous.

With subject permission, we may store specimen data with or without subject's name or identifying information for future use. Retention of blood specimens after the end of the study for use in future research must be granted permission from the subject on the ICF. Subjects will give permission on the ICF if they agree to keep their name and other identifying information with their specimens. See 11.4 for future use of stored specimens plan.

13 RESULTS:

13.1 Feasibility: This was a feasibility study, initially powered for a sample size of 44. We fell far below the recruitment goals, despite 2 years of recruitment, finding a lack of interest for this intervention from the desired patient population. Therefore we consider the feasibility goal to have been a failure. The results should be interpreted with caution as the study was likely underpowered and the real world acceptability of the intervention in the context of this study was not desirable enough to recruit participants.

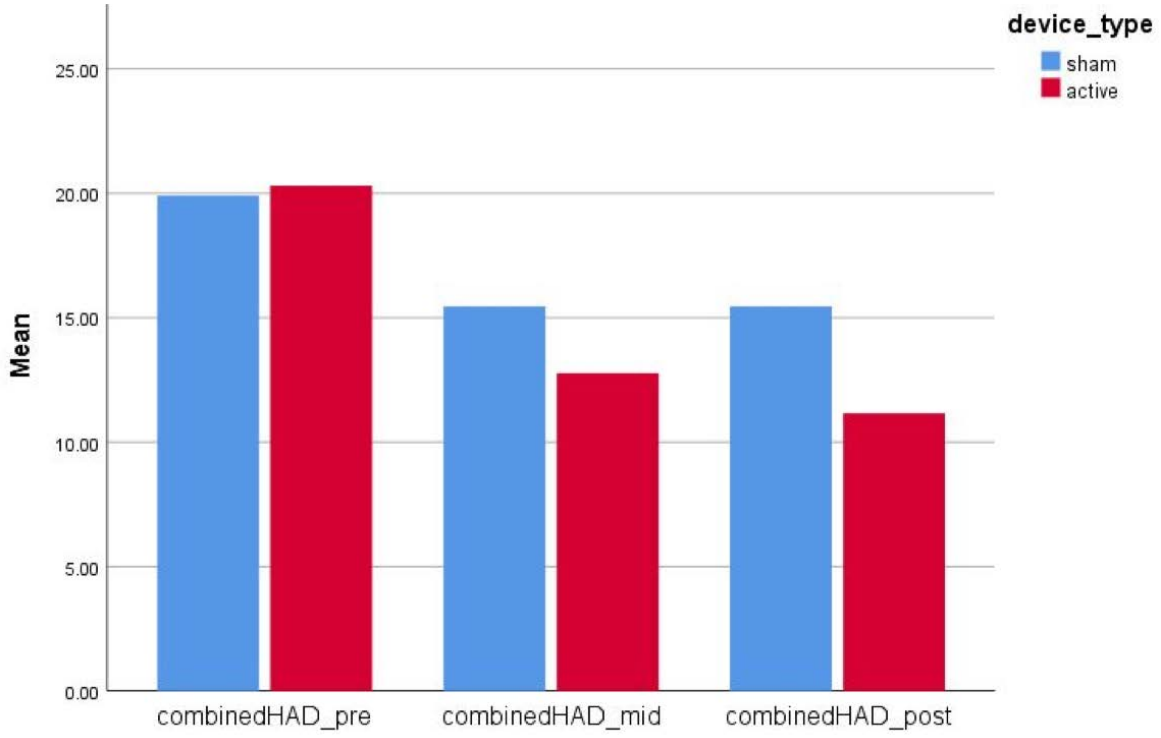
13.2 Baseline values and demographics:

11 subjects completed the Sham treatment and 13 completed the active treatment. No baseline differences in age, body mass index, HAD anxiety, HAD depression, or telomere length were identified between subjects randomized to the sham or active device groups. Both groups had 5 Veterans. Two subjects did not have baseline BMI measured. 1 subject did not have sufficient sample to complete the Telomere length.

No baseline differences were noted in age, BMI, anxiety, depression between Veterans and non-Veterans (all $p>.05$)

13.3 Primary outcome: change in combined HAD score

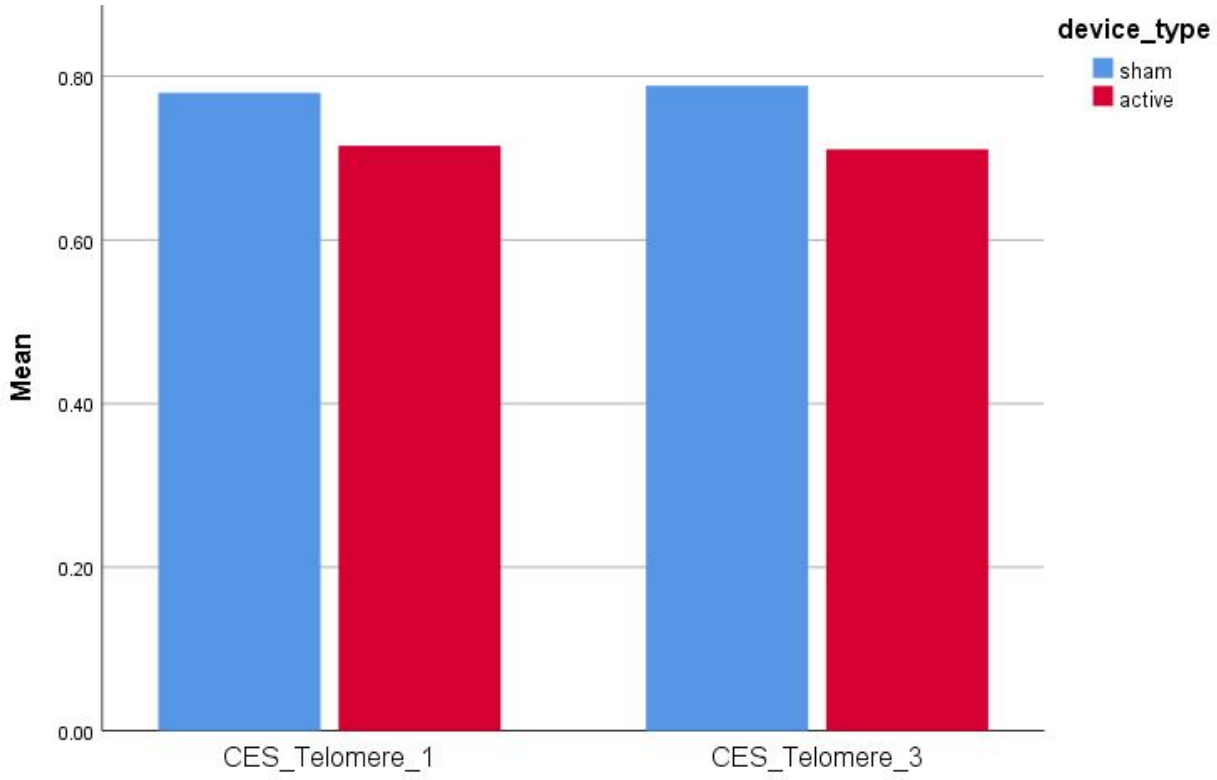
The Active treatment group had a decrease in combined HAD score of 8.8 and in the Sham group the decrease was 3.64 (shown in table); t=-2.32, p=.013.



13.4 Secondary outcome: Telomeres

13.4.1 Telomere length

No differences in telomere length were noted in pre to post treatment phase,p=.33.



13.4.2 Telomerase activity

The results of the telomerase assay showed unacceptably high intra and inter-subject variability, multiple outliers, and non-normal distribution (baseline values skewness 3.1, kurtosis 10.7; end values skewness 2.1, kurtosis 4.1). It is unclear whether this was secondary to natural population variations or assay unreliability but it was determined that analysis of this small sample was unlikely to result in an interpretable finding.

13.5 Exploratory outcomes

These outcomes were not measured due to the lower than expected sample size.

14 CONCLUSIONS AND INTERPRETATION

The study was considered a feasibility failure due to failure to recruit subjects in general and Veteran subjects in particular. There are multiple potential reasons for this. First, the study site was not located at the Veterans clinic, it was at UCLA which requires 15-30 minutes drive or bus ride. The study compensation was low for the time that the participants were involved. There was not an advertising budget to reach a larger group of eligible individuals. The treatment interventions required an hour of use per day which may have been unacceptable to this young population.

Analysis of the anxiety and depression combined score showed a small benefit of the active over the sham device. This is consistent with other studies and supports the possibility that the intervention has benefit on negative affect in young men. Larger studies will be required to confirm this.

Analysis of the telomere lengths did not show differences between subject groups. Due to the small sample this is hard to interpret, but may represent a lack of effect of mildly improved mood on telomere length in young men.

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SUPPLEMENTAL MATERIALS

- Phone Prescreening Script
- Informed Consent Form
- HIPAA Authorization
- Mini International Neuropsychiatric Interview (MINI)
- Hospital Anxiety and Depression Scale (HADS)
- Mental Health Continuum Short Form (MHC-SF)
- Patient Reported Outcomes Measurement Information System (PROMIS-29)
- PTSD Check List - Military (PCL-M)
- Positive and Negative Affect Schedule Short Form (PANAS-SF)
- Connor-Davidson Resilience Scale (CD-RISC)
- Perceived Stress Scale (PSS)
- Adverse Childhood Experiences (ACE)
- Patient Health Questionnaire (PHQ)
- Columbia-Suicide Severity Rating Scale (C-SSRS) Self Report Screener – Recent
- Columbia-Suicide Severity Rating Scale (C-SSRS) Self Report Screener – Since Last Contact
- Expectancy for Improvement Scale
- Impression of Global Improvement Scale
- Tracking Diary
- Instructions for Daily Use
- Alpha-Stim Brochure
- CES Requisition Form
- Internet Ad Posting
- Internet Ad Flyer
- Tear-Off Flyer