Study Protocol

Study: Vagus Nerve Stimulation: Integration of Behavior and Cardiac Modulation

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PARTNERS HUMAN RESEARCH COMMITTEE
DETAILED PROTOCOL

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Title: **Vagus Nerve Stimulation: Integration of Behavior and Cardiac Modulation**

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I. BACKGROUND AND SIGNIFICANCE

Implantable VNS (iVNS) has been safely used to treat thousands of patients with depression. Eighty percent of the fibers in the vagus nerve are afferents that project to the periaqueductal grey (PAG), nucleus tractus solitarius, limbic system and thalamus. Secondarily, there are projections to the prefrontal cortex, inferior and superior parietal cortex and basal ganglia. Imaging studies in MDD patients have confirmed the broad reach of vagal afferents. In depressed patients, iVNS/fMRI interleaving activated bilateral orbitofrontal cortex, parieto-occiptal cortex, hypothalamus, left amygdala and globus pallidus, areas implicated in the pathophysiology of MDD.

However, iVNS has side effects that limit its attractiveness as a treatment. The location of the stimulating electrode is within the vicinity of the recurrent laryngeal nerve, thus can cause hoarseness during stimulation, i.e. every 5 minutes. In addition, it is an invasive procedure with associated anesthesia and surgical risks. Transcutaneous VNS (tVNS) (placed in the ear) is an alternative that has been studied in healthy subjects. The auricular branch of the vagus nerve carries only vagal afferents unlike the vagal branch used in iVNS, which contains 20% efferents to the heart. Stimulating the auricular branch thus carries no risk of direct suppression of heart *rate*. tVNS is a non-invasive procedure that can be easily removed from the ear. Studies have shown that tVNS stimulates overlapping neural networks in healthy controls as iVNS in depressed patients, including hippocampus, amygdala, anterior cingulate, insula and thalamus. Dr. Vitaly Napadow, collaborator, has demonstrated that tVNS decreases anxiety symptoms in women with chronic pain, a symptom highly comorbid in depression. Therefore, tVNS may be a lower risk and effective means of treating patients with MDD. Recently, a pilot study of tVNS was performed in depressed patients and demonstrated significant decreases in depressive symptoms compared to sham.

The circuits stimulated by VNS (i.e. mood circuitry) overlap with the circuits for autonomic nervous system (ANS) regulation. Heart rate variability (HRV) is an important measure of the health of ANS. HRV analysis indirectly estimates sympathetic and parasympathetic modulation of the heart. The peak in low-frequency spectral band (LF) is thought to be influenced by both parasympathetic and sympathetic activity. The peak in high-frequency band (HF) is influenced solely by parasympathetic (vagal) activity and the LF/HF ratio approximates the balance between sympathetic and parasympathetic modulation of the heart. Deficits in vagal tone have been found in patients with MDD as evidenced by decreased HF band. Vagal output from the hypothalamus projects to the heart and releases acetylcholine to slow heart rate. Vagal afferents provide feedback regulation of the heart through the nucleus tractus solitaries, which then projects to the heart. This feedback on heart rate can come from any of the vagal afferent branches. The auricular branch of the vagus nerve is an easily accessible vagus branch that can be directly stimulated. The dorsal medullary vagal system operates in tune with respiration, and therefore stimulation gated to exhalation/inhalation might have modulatory effects on vagal tone. The systematic

study of respiratory-gated tVNS in MDD patients may reveal whether in addition to regulating mood, it can regulate HRV.

Although deficits in vagal tone have been found in MDD, VNS has not yet been used in MDD patients to improve vagal tone. There have been reports of bradycardia and even asystole during implantation of iVNS. This may be due to direct stimulation of the vagal efferents of the target branch of the vagus. Although cardio-depressant effects have not been seen post operatively during active iVNS stimulation, modulation of vagal tone after iVNS has been reported. Complex studies of heart rate and the influences of vagal tone have not been performed in MDD with tVNS. Acupressure to the auricular branch of the vagus nerve restored sleep in post menopausal women. More importantly, the effect was only found in women who also had an increase in HF-HRV. If chronic tVNS normalizes HRV in MDD, it has the potential to have a significant impact on public health considering the high comorbidity between MDD and cardiovascular disease. Deficits in vagal tone have been associated with the comorbidity of MDD and CVD. Modulation of HRV chronically in MDD may thereby decrease risk for cardiac morbidity and mortality.

II. SPECIFIC AIMS

Major Depressive Disorder (MDD) is a leading cause of morbidity and mortality worldwide. Abnormalities in vagal tone are more common in MDD, particularly loss of parasympathetic cardiac regulation, and increase the risk for cardiovascular disease (CVD). Vagal nerve stimulation (VNS) is an FDA approved treatment for MDD that modulates circuitry implicated in mood regulation. However it is unclear whether therapeutic benefit of VNS on mood, can also reduce cardiac morbidity and mortality. The circuits for autonomic cardiac regulation overlap with those for mood regulation, but modulation of ANS has not been directly associated with mood response. As a first step, we will identify whether VNS effects on mood circuitry in MDD are associated with modulation of vagal tone and mood.

In the proposed study in MDD patients, we will 1) identify the neural circuitry associated with tVNS, 2) identify its acute effects on heart rate variability, and 3) assess the impact of brain activity and ANS function on mood. We will control for factors known to impact vagal tone, in particular gonadal hormones in women. This is the first study to map the neurobiology of mood and vagal response to VNS using a less invasive form of VNS that may have a wider application.

Specific Aim 1: To determine the acute effects tVNS on cerebral blood flow in MDD patients.
Hypothesis 1: During real tVNS/fMRI interleaving, tVNS will deactivate amygdala, parahippocampal gyrus, hippocampus, posterior cingulate, and precuneus and will activate insula, thalamus and anterior cingulate.
Hypothesis 2: Brain activity deficits in stress response circuitry in MDD will be attenuated following 20 minutes of tVNS, in hypothalamus, amygdala, hippocampus, and anterior cingulate gyrus.
Specific Aim 2: To determine the acute effects of tVNS on vagal tone and mood in MDD patients.
Hypothesis 1: tVNS will increase high frequency R-R variability and decrease mood symptoms compared to baseline.
Specific Aim 3: To compare the effects of exhalation-gated tVNS with inhalation-gated tVNS on cerebral blood flow, vagal tone and mood regulation in MDD patients.
Hypothesis 1: Exhalation-gated tVNS will have a significant greater impact on regulation of cerebral blood flow, vagal tone and mood in MDD patients compared to inhalation-gated tVNS.

III. SUBJECT SELECTION

About 25 female, right-handed subjects will take part in this research study at Brigham and Women's Hospital (BWH). (Up to) the first 5 subjects enrolled in the study will not have major depressive disorder (MDD). These non-MDD (test) subjects will do some of the study procedures so that we can make any changes before we enroll subjects with major depression. Following finalization of study procedures, up to 20 female subjects with MDD will be enrolled. Subjects with major depression will participate in 2 telephone screenings and/or a REDCap online questionnaire, an evaluation visit, two MRI testing sessions, and four follow-up evaluations. The non-MDD test subjects will only participate in the telephone screening and one MRI session.

Inclusion Criteria/Exclusion Criteria:

Inclusion Criteria

Patients with MDD will be women between 25-40 years of age meeting DSM criteria of recurrent MDD as assessed by SCID. In addition, up to five non-MDD subjects will be enrolled to test all procedures prior to enrollment of MDD subjects. MDD patients with a comorbidity of PTSD will be considered for clinical applicability by our senior clinicians on a case by case basis.

Exclusion Criteria

- History of Axis I psychiatric diagnosis other than MDD or anxiety disorder - e.g., substance use disorder, psychotic disorder, or bipolar disorder.
- Current Suicidal Ideation with intent and/or plan or history of suicide attempt within the last year
- Use of psychotropic medications within four weeks prior to study with the exception of SSRI and SNRI class of antidpressant medication only
- Use of Tricyclic antidepressants (TCAs), Monoamine oxidase inhibitors (MAOIs), and Atypical agents
- History of cardiovascular disease
- History of neuroleptic use
- Past history of substance abuse or dependence within the past 12 months (excludes nicotine)
- Bleeding disorder or use of anticoagulants.
- Pregnancy
- Metallic implants or devices contraindicating magnetic resonance imaging.
- Use of beta blockers

Verbal Screening

Women of childbearing age will be asked if they think they are pregnant and if they are trying to get pregnant. Upon self-report, if they are not sure whether they are pregnant, OR if they are trying to get pregnant, then they will not be allowed to participate in this study. Negative pregnancy test allows study entrance.

Source of subjects and recruitment methods

Healthy adult female volunteers with MDD (and up to five test subject volunteers without MDD) will be recruited by poster, e-mail and website advertisements (see attached flier), phone call (with prior consent to contact), as well as from the outpatient psychiatry clinic and the registry Research Study Volunteer Program (RSVP for Health). The research assistant will first perform a phone interview with the subjects where an initial screening will take place to distinguish potential subjects from those not meeting eligibility criteria (as detailed above). If a potential subject responds to the REDCap online survey and meets eligibility criteria, the research assistant will contact the subject for further scheduling and to answer any questions. Efforts will be made to attain a mix of study participants in terms of racial/ethnic representation as determined by NIH guidelines. Children will not be included because of differences in

brain development with age. It would also be difficult for them to lie still throughout the scanning period. Patients will be recruited with the help of outpatient psychiatrists and colleagues.

IV. SUBJECT ENROLLMENT

Method of enrollment

When contacted by a potential volunteer, the research assistant will either first perform an initial screening interview via phone. This will be done to distinguish potential subjects from those not meeting eligibility criteria. During this initial interview, we will explain the purpose, procedures, and potential discomforts/risks of the study. If a potential subject responds to the REDCap online questionnaire and meets eligibility criteria, the research assistant will contact the subject to schedule her for a second phone screening and to answer any questions. If the subject is eligible by the initial phone screening or REDCap online questionnaire, a clinician Interviewer will have a second phone screening interview. This second phone screening interview will help determine if the subject is eligible for an in person evaluation. On the day of the evaluation, MDD subjects will again be explained the purpose, procedures, and potential discomforts/risks of the study. They will again undergo a deeper screening procedure and will be asked about their medical and psychiatric history and fill out a handedness questionnaire to assess that they are right handed (see attached forms/questionnaires). The evaluation is expected to take about 90 minutes. Only English speaking subjects will be enrolled as we do not have a Spanish speaking clinician to perform assessments. Non-MDD test subjects will only participate in the telephone screening and one MRI session, and thus will not participate in the fill screening appointment.

Procedure for obtaining informed consent

After the initial phone screenings detailed above the post doctoral fellow will explain the purpose, procedures, and potential discomforts/risks of the study and then obtain a signed informed consent from the prospective subject.

Subjects will be paid $25 for the Clinical Evaluation, $150 for each MRI appointment in which they participate and $25 for a telephone follow-up evaluation. If scanning is stopped due to subject discomfort, technical problems, or if a subject is found ineligible prior to entering the scanner, they will still be compensated for their visit.

Confidentiality

To respect confidentiality and the HIPAA rules, the subject's name is not stored with their data. All the participant's identities and data will be held in confidential files. On entry to the study subjects will be assigned a study ID#, and all files will use this ID for future references. All information regarding experimental subjects will be kept in a locked file cabinet in the office of the principal investigator. All study staff are IRB certified and all information and records are in compliance with HIPAA rules.

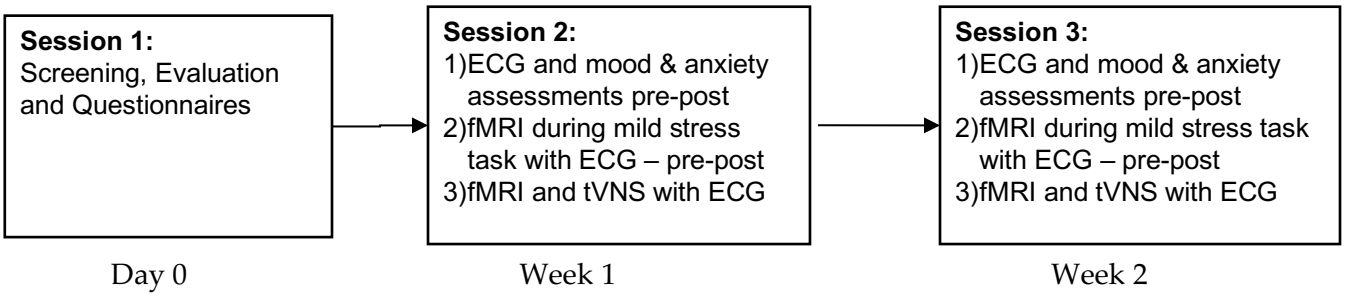
V. STUDY PROCEDURES

Study visits and parameters to be measured

Experimental Design Overview

The fMRI scanning session will take approximately 90 minutes. There are 2 conditions, i.e. two scanning sessions *(note: 5 non-MDD test subjects have one scanning session only)*. Both conditions will consist of one 30 minute tVNS session, one inhalation-gated the other exhalation-gated. During both

conditions, we will perform electrical stimulation to the ear, to stimulate the auricular branch of the vagus. Stimulation parameters will be identical.



tVNS Procedures for all Scans

Periods of tVNS (inhalation-gated vs. exhalation-gated) stimulation will alternate with periods of rest, during which time the electrodes will remain in place.

tVNS will be performed at the left ear by either Dr. Vitaly Napadow (trained in tVNS) or the post-doctoral fellow. Two reusable MRI compatible electrodes will be attached to the cymba and cavum concha (**Figure 2**) of the auricle. Current will be delivered with UROstim transcutaneous electrical stimulator (Schwa Medico, Germany). Current amplitude will be set between the sensitivity threshold and the pain threshold for each subject. The waveform will consist of monophasic rectangular pulses. The stimulation parameters will include 30 Hz stimuli with 300 μsec pulse width. The output current will be increased gradually to a maximum of 8 mA (in 0.25-mA increments) to allow accommodation to the stimulation until a comfortable tolerance level is reached. Stimulation will be on for 30 seconds and off for 30 seconds during the fMRI scan runs. These stimulation parameters and procedures are similar to those used in iVNS for depression. Respiratory gating for stimulation will require real-time evaluation of the respiratory cycle. An adaptive threshold detection method will be employed to detect end-inspiration and end-expiration in real-time and provide a signal controlling the delivery of the stimuli during the exhalatory or inhalatory pahse of respiration. Correct respiratory-gated stimulation will be confirmed by the experimenter via running chart of the respiration signal and stimulus pulse.

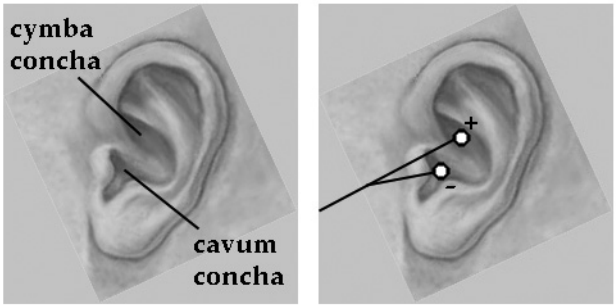


Figure 2 – tVNS electrode placement

Since receptors respond directly to current load and normal tissue response alters electrical resistance, it is important to use a current-constant EA stimulation device. A voltage-constant device (common in clinical practice) produces variable current load, and leads to variations in stimulation intensity.

Clinical Assessments

Outcomes will include whether acute stimulation with tVNS reduces mood symptoms. Our group has found tVNS diminishes anxiety in an acute paradigm. Measures at intake will include: Structured Clinical Interview for DSM Disorders, Hamilton Rating Scale for Depression 17, and the Montgomery Asperger Depression Rating Scale. Before and after tVNS/fMRI, we will assess mood with the Beck Depression Inventory-II, Hamilton Rating Scale for Depression 17, Spielberger State-Trait Anxiety Interview, and a Visual Analog Scale.

Blood Draw

Our on-site Phlebotomist will draw one sample [(approximately 5 teaspoons (26 ml)] at the beginning and another blood sample [approximately 3 teaspoons (13 ml)] after each of the two imaging visits. Previous studies from our group have demonstrated gonadal hormonal regulation of mood and autonomic nervous system circuitry. In addition, a significant body of evidence has suggested an interrelationship between the activity of the autonomic nervous system, particularly the vagus nerve and the immune system. We will use estradiol, progesterone, cytokines (IL-1β, IL-6, IL-10, TNFα), and C-reactive protein (CRP) levels as covariates in the analysis of tVNS effects in the activation of brain regions related with mood and autonomic modulation. The results of the blood tests are not clinically relevant to individual persons; therefore, we will not return results to the subject. However, when the study analyses are complete, we will make study results available to the subject, if desired (although individuals are not identified in these analyses).

Saliva Collection

We would like to collect a sample of saliva (15 ml saliva tube) to examine cortisol levels in order to evaluate the state of the hypothalamic-pituitary-adrenal (HPA)-axis in depressed subjects. Collection will take place the evening before the scan (11pm), prior to the scan, and 60 minutes following the picture task. This variable will be considered in the analysis of the effects of our intervention in the modulation of the stress response circuitry and central autonomic network. The samples will have no personal identifying information and will be placed in a storage bank in secure buildings in the Boston medical area that have limited access. They will be used only for research that is carefully reviewed and approved both by this study and the Institutional Review Board.

Scanning

Subjects will be randomized as to the order of scan dates (inhalation-gated vs. exhalation-gated), which will be separated by at least one week. Scanning begins with the subject at full rest then they will undergo our mild visual stress paradigm for 18 minutes. Then the electrodes will be placed. The stimulation block will have a total duration of 30 minutes. The electrodes will be withdrawn at 20 minutes after and the mild visual stress task repeated. Patients will first undergo a mild stress task where they view pictures during fMRI scanning. Our fMRI stress paradigm has been described in detail previously and conducted in healthy subjects, MDD, and psychotic cases. Briefly, subjects view 3 stimulus blocks (6 images per block) for 30 seconds (1 image every 5 seconds) in the following order: fixation, neutral, and negative images (see definitions below). Subjects view this sequence 4 times during each of 3 runs for a total of 72 images (24 images per condition per run), and press a button each time a new image appears in order to ensure attention to the images, without adding cognitive load. Images were drawn from the International Affective Picture System (IAPS) according to affective valence and arousal (negative = unpleasant + high arousal, neutral = neutral + low arousal). Fixation images are based on Fourier transformations of each neutral image to create an image with the same physical properties of the original but without recognizable content. The stress task will be repeated after the tVNS portion of imaging. When the fMRI session is complete, subjects will be shown two blocks of negative and neutral images sampled from the set shown during imaging and will be asked to provide subjective ratings of arousal using the Self-Assessment Manikin (SAM).

Post-Scan Follow Ups

After each fMRI scan we will follow up with each subject to assess their mood. We will follow up one day and seven days after each scan. The follow-ups one day after the scan will consist in the administration of a Visual Analogue Scale for mood states through a REDcap online survey. For the 7th day follow-ups we will administer the Hamilton Depression Scale, Beck Depression Inventory-II, Spielberger State-Trait Anxiety Interview, and the Visual Analog Scale. The first 7th day follow up will be conducted in person at Imaging Visit #2. The second 7th day follow up will be conducted by phone. These follow ups will evaluate acute changes in mood following tVNS administration during the imaging visits.

Acquisition Parameters for FMRI and Physiological Data Collection

General MRI Scanning Procedure for Subjects: When the subject arrives for the MRI scan, she will be asked to fill out a brief questionnaire in order to confirm that they can safely enter the MRI scanner. She will be asked to remove any metallic items from her body and may be given non-metallic shirts and pants to wear in the scanning room. The participants will be instructed to lie on the MRI table. Once the participant is positioned comfortably on the MRI table, the table will be moved into the MRI scanner tube. The participant will be able to communicate with the operator outside of the MRI room. The participant will be asked to lie still during imaging. To help immobilize the participant's head, foam pillows will be placed under and around the head. Subjects may be asked to use a "bite bar" which conforms to the teeth, or a plastic mask that conforms to the face, to help keep the head still. Since the MRI scanner makes loud knocking or beeping sounds during the scan, the participant will be asked to wear earplugs during imaging. If the participant becomes uncomfortable at any time, the scan will be stopped and the problem eliminated. If it is not possible to alleviate the problem the scan will stopped and the participant will be free to discontinue participation.

Structural MRI: To provide anatomical localization of the functional signal, high-resolution (voxel size= 1 mm^3) structural MRI scans will be acquired using a T1-weighted MP-RAGE pulse sequence (TR= 2300 ms, TE= 2.95 ms, acceleration= GRAPPA factor 2, flip angle= 9º, FOV= 256 x 256 mm^2, 176 axial slices).

BOLD imaging: Whole-brain fMRI data will be acquired with gradient-echo echo-planar imaging (EPI) using Simultaneous Multi-Slice (SMS) acquisition with multi-band factor 5 (TR= 1250ms, TE= 33 ms, flip angle= 65º, FOV= 200 x 200 mm^2, 75 axial slices, voxel size= 2 mm^3).

Physiological Monitoring During fMRI Scanning: Peripheral physiological data will be acquired using a Powerlab system (ML880; ADInstruments Inc, Colorado Springs, CO) at a 1000 Hz sampling rate. We will use a magnetic resonance-compatible pneumatic belt placed around the subject's lower thorax to evaluate changes in respiratory volume. In addition, cardiac pulsatility data will be acquired using a piezoelectric pulse transducer (AD instruments Inc., Colorado Springs, CO) attached to the right index finger.

Data Storage

Any data obtained from subjects during this study will not leave our institute in any form that would identify individual subjects. All subject data will be identified by a subject ID number. The PI will keep a master list of subject names and corresponding subject ID numbers in a locked cabinet at the BWH. Electronic data will be housed in secure, password protected computer accounts.

VI. BIOSTATISTICAL ANALYSIS

Specific data variables being collected for the study

For this study data will be collected from MRI in electronic form. Subjects will also provide data in the form of questionnaires related to mood and medical history. Copies of these subject forms/questionnaires are included with this application.

Sample Size (Power Analysis)

The present study will recruit a total of 20 subjects (women age 25-40 with recurrent MDD). This will allow for the possibility of unusable data due to subject motion during scanning, scanner artifact problems as well as problems with the collection of behavioral data and human error.

Group size power statistics: In a previous study of manual acupuncture (N = 11), we were able to detect changes in activation of limbic and subcortical gray structures in the approximate range of -0.7% to -0.2%.

Also based on previous data (amygdala, manual acupuncture stimulation), we will assume a standard deviation of BOLD signal change of 2.5% for the purpose of power calculation and sample size determination. We do not consider temporal autocorrelation in the power calculations that follow, though we will take autocorrelation into account during the data analysis.

Statistical Analysis and Methods

Behavioral Outcomes analysis

The Beck Depression Inventory-II and the Spielberger State-Trait Anxiety Inventory will be used to evaluate acute changes in mood and anxiety symptoms. Behavioral data will be analyzed using repeated measures ANOVA [mood and anxiety ratings by time (PRE-, POST-stimulation) and session (exhalation-gated, inhalation-gated)], controlled for baseline.

Magnetic resonance imaging data analysis

Comparisons of interest in the fMRI analysis (BOLD signal changes during exposure to negative vs neutral images) will be tested using linear contrasts and results from the individual subject level will be submitted to a second-level factorial analysis with two factors: time of visual stress task (PRE- or POST-stimulation) and stimulation session (exhalation-gated or inhalation-gated). Significant interactions between these factors will be submitted to post-hoc analyses to determine directionality of the effect. Due to our a priori hypothesis of specific effects of tVNS on stress response circuitry, the analysis will be restricted to specific regions of interest (ROIs) using a small volume correction approach. A voxel-wise height threshold of $p<0.001$ (uncorrected for multiple comparisons), and a cluster correction with FWE p-value<0.05 will be applied. Mean beta weights within each significant cluster will be extracted for each participant using the REX toolbox and will be included in regression analyses in STATA (StataCorp, College Station TX) to investigate the link between variations in stress response circuitry activity and changes in depressive symptoms (BDI score difference: POST-PRE) after tVNS administration. Adjusted regression coefficients will be calculated after including baseline BDI scores and use of antidepressant medications in the models.

Functional connectivity will be evaluated during tVNS stimulation runs by extracting fMRI time series from the selected ROIs and using them in a weighted GLM and semi-partial correlation analyses. Resultant whole-brain parameter estimates and their variance from each individual will be passed to group-level analyses to evaluate connectivity differences between exhalatory- and inhalatory-gated sessions. For evaluation of functional connectivity during IAPS, the extracted fMRI time series from the selected ROIs and its interaction with the regressors for negative and neutral content will be used to evaluate connectivity differences between the visual stress tasks during PRE- and POST-stimulation, for exhalatory- and inhalatory-gated sessions. General linear models (GLM) will be conducted in STATA (StataCorp, College Station TX) to investigate the link between variations in functional connectivity and changes in depressive symptoms in response to tVNS administration Adjusted regression coefficients will be calculated after including baseline BDI scores and use of antidepressant medications in the models.

Cardiac Autonomic Analysis

A point-process algorithm will be used to separate the heart rate variability dynamics in the classic spectral component within the high-frequency (HF) and low frequency (LF) ranges. Differences in normalized HF [HFn= (HF/(LF + HF))] will be estimated during exposure to negative images in the fMRI stress task as a metric of cardiac vagal activity.

VII. RISKS AND DISCOMFORTS

Blood Draw

Subjects may feel slight discomfort when we insert the needle and draw blood through a vein. Very rarely, the vein may become sore and red. In addition, a temporary, harmless bruise may develop. Although rare, some people faint when blood is drawn. In rare cases, the subject can get an infection where the blood was drawn. The infection can usually be treated with antibiotics.

Saliva Collection

At this time, there are no known risks for the saliva collection.

MRI Scanning

There are no known or foreseeable hazards or risks associated with MRI imaging except for people with electrically, magnetically, or mechanically activated implants (such as cardiac pacemakers) or those who have metallic clips in their brain – all of whom would be excluded from this study. The powerful magnetic field created by the MRI can disturb these metal objects. In addition, since the effect of MRI on the fetus is unknown, women suspect pregnancy will be excluded from the study. The investigator will ask the participant if they are prone to claustrophobia, if so they will not be scanned. The participant will be asked to fill out a screening questionnaire prior to the MRI scan in order to rule out any condition that may preclude MRI scanning. Conventional MRI systems have been approved by the FDA and will be operated within the operating parameters reviewed and accepted by the FDA. The loud gradient noise if the MRI could potentially cause hearing damage if no protection were to be used, therefore, all subjects are required to wear earplugs to ensure scanner noise is reduced to safe levels. During the scan, voice contact with participants will be maintained. Participants will be instructed to notify the examiner of any discomfort. If the discomfort cannot be alleviated, the scanning session may be terminated prematurely and inquiries will be made to assess if subjects are well and comfortable.

MRI could potentially uncover an anatomic abnormality in the brain. If this occurs, the established protocol for the BWH Research Imaging Center will be followed. If any anatomic abnormality is suspected in the anatomic MRI images, the films will be reviewed by the staff radiologist in the scanning facility. The radiologist will decide whether the suspected abnormality is clinically significant and will discuss the findings as well as further recommendations with the participant.

tVNS Stimulation

There is a small possibility of slight and temporary discomfort at the site where the electrodes are placed. Potential vaso-vagal reactions to stimulation resulting in dizziness or light-headedness will be promptly recognized and treated by a study physician with prompt removal of the electrodes and repositioning the subject into a supine position. In some subjects, the experience of electrical stimulation may cause anxiety. Subjects will be required to remain under supervision until all symptoms subside. A study physician will be available at all times to discuss the study with subjects should they become concerned.

In the proposed study, only non-magnetic electrodes will be used. The electrical stimulation procedure is without significant safety concerns. The electrical stimulator, checked by Biomedical Engineering, has a current limiter preventing harmful stimulation levels. Further, during the electrical stimulation, we will be closely monitoring that the current received by the subject is well tolerated. Throughout the measurement sessions, participants will be repeatedly asked if they are well and comfortable. Should the subject feel any discomfort in the fMRI measurements, she will be advised to squeeze the pneumatic signaling device (always held in her/his left hand) and the measurement will be immediately interrupted.

VIII. POTENTIAL BENEFITS

There are no expected benefits for subjects participating in these studies. However, the findings of this investigation may help advance the field of developing novel therapeutics as well as our understanding of pathways that may mediate the body's mood and cardiac mechanisms. Therefore, we believe that the slight risks associated with these studies are reasonable in relation to the potential benefits.

IX. MONITORING AND QUALITY ASSURANCE

The investigator in charge of the study will perform safety monitoring during the entire imaging session. The PI and co-investigators will also review the data for consistency and accuracy as well as monitor that analysis is conducted properly. Furthermore, the technical staff at the Longwood Imaging Center will continually monitor the nature and quality of the data collected using the 3T MRI scanner. The participants data will be stored in a computer network in a secure, password protected, account. The participant will be identified by a unique participant number that is linked only to their name by a master data sheet that is kept in a secure locked filing cabinet.

In the case of a serious adverse event (i.e. death, life threatening experience) the incident will be reported immediately (within 24 hours of event) by telephone, fax or e-mail followed by a full written report using the PHRC Adverse Event Form within 10 working days/14 calendar days. In the case of a mild (i.e. minor irritant type) or moderate (i.e. discomfort requiring treatment) adverse event that is unexpected but may be related to the experiment, the incident will be reported in writing using the PHRC Adverse Event Form within 20 working days/30 calendar days. In the case of a mild or moderate adverse event that is unexpected, but not related to the experiment, the PI will summarize the event in the progress report at continuing review. In the case of a mild or moderate adverse event that is expected the event will be summarized by the PI in the progress report at continuing review.