A Randomized Control Trial Treating Depression With Yoga and Coherent Breathing Versus Walking in Veterans

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HUMAN SUBJECTS RESEARCH
PROTOCOL

Project Title: A Randomized Controlled Trial Treating Depression with Yoga and Coherent Breathing Versus Walking in Veterans

Short Title: RCT Treating Depression with Yoga or Walking in Veterans

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Institution(s): The VA Bedford Healthcare System (VABHCS) is the coordinating site. The McLean Hospital, Belmont MA will be the site for MRI imaging.

1.0 OBJECTIVE AND SPECIFIC AIMS:

Aim 1: To determine in Veterans, the effectiveness of an RCT of 12-weeks of the yoga intervention versus walking intervention, with depression and mood assessed at baseline prior to randomization, and prior to and immediately following yoga or walking interventions at weeks 4, 8, and 12, and before each scan.
Hypothesis I: In this working clinical hypothesis, subjects in the yoga group will exhibit greater improvements in depressive symptoms and mood ratings over the study compared to the walking group.

Aim 2: To measure changes in thalamic GABA levels at Scans 1, 2, and 3. (3)
Hypothesis II: In this working translational hypothesis, GABA levels will increase and depressive symptoms will decrease over the course of the 12-week intervention in the yoga group compared to the walking group.

Aim 3: To examine changes in RSA acquired at baseline prior to randomization, and prior to and immediately following yoga or walking interventions at weeks 4, 8, and 12, and before each scan.
Hypothesis III: In this working translational hypothesis, RSA levels will increase and depressive symptoms will decrease over the course of the 12-week intervention in the yoga group compared to the walking group.

Exploratory Aim: If an individual has symptoms of Post Traumatic Stress Disorder (PTSD) at screening, there will be a greater decline in PTSD symptoms with treatment in the yoga compared to the walking group. The use of the 3T scanner allows the collection of pre and post exploratory data for cortical thickness, resting state, and executive function.

2.0 BACKGROUND AND SIGNIFICANCE

2.1 Background Cost of Major Depressive Disorder (MDD): The World Health Organization Global Burden of Disease study estimated that depression is the single most burdensome illness during the middle years of life, in both developing and developed countries, with no other disease accounting for even half of the total burden.(1) Advances in the treatment of MDD include antidepressants, psychotherapy, and somatic treatments such as Electroconvulsive Therapy (ECT), Vagal Nerve Stimulation (VNS) and Transcranial Magnetic Stimulation (TMS). Although these treatments have been found effective for reducing symptoms of

MDD in RCTs, 40 to 50% of individuals with MDD do not achieve remission.(2,3) Even the Sequenced

Treatment Alternatives to Relieve Depression (STAR*D) study of non-psychotic MDD subjects, which has 4 treatment steps for advancing individuals who fail medication trials, has an overall cumulative remission rate of only 67%.(2, 4) Notably, individuals who have one depressive episode are at increased risk for another depressive episode. However the risk of recurrence is significantly lower in 61% of individuals with asymptomatic recovery from a Major Depressive Episode, and they remain free of a relapse or recurrence 4.2 times longer than those who continue to exhibit subsyndromal depressive symptoms.(3) The use of yoga-based therapies as monotherapy or adjunctive treatment for depression represents a promising area of research to address the current short falls in available therapies.

2.1.a. **Vagal-GABA Theory [Figure 1]:** The Vagal-GABA theory developed by our group, explains why yoga practices should benefit numerous comorbid disorders that are common in the VA population, by increasing activity in parasympathetic and GABA systems. It is hypothesized that stress induces: 1) imbalance of the ANS, with decreased parasympathetic nervous system (PNS) and increased sympathetic nervous system activity (SNS), 2) an underactive GABA system, and 3) increased depressive symptoms. It is further hypothesized that yoga and coherent breathing correct underactive PNS and GABA systems in part through stimulation of vagal nerves, reducing allostatic load caused by stress and decreasing depressive symptoms.

2.1.b. **How Yoga Affects the Autonomic Nervous System:** According to the Polyvagal Theory developed by Porges (1995), the myelinated vagus, the most evolutionarily advanced component of the ANS, supports social engagement, engenders feelings of safety, and promotes calm states consistent with the metabolic demands of growth, repair, and restoration.(5) Respiratory Sinus Arrhythmia (RSA) is a validated measure of myelinated vagal efferent out flow from the nucleus ambiguous to the sino-atrial node.(5) RSA represents only the myelinated vagal contribution to cardiac vagal tone, as indicated by the high frequency (HF) component of heart rate variability (HF-HRV). Increases in RSA reflect increases in parasympathetic activity and vagal dominance.(5) The proposed study will measure RSA because it is specific to the myelinated vagal contributions to changes in heart rate, thus excluding non-vagal contributions to changes in HRV.(5) The sequence of evolutionary development ascends from the unmyelinated vagus to the SNS and, most recently inmammals only, the myelinated vagus. The myelinated vagus conducts impulses more rapidly than the SNS and the unmyelinated vagus. Myelinated vagal efferent fibers from the nucleus ambiguous serve as the vagal brake, enabling rapid control of heart rate by increasing vagal tone (stepping on the brake) to reduce heart rate or decreasing vagal tone (letting up on the brake) to accelerate heart rate. The vagal nerve, Cranial Nerve X, is the primary parasympathetic pathway of the peripheral nervous system. Inspiration and expiration,

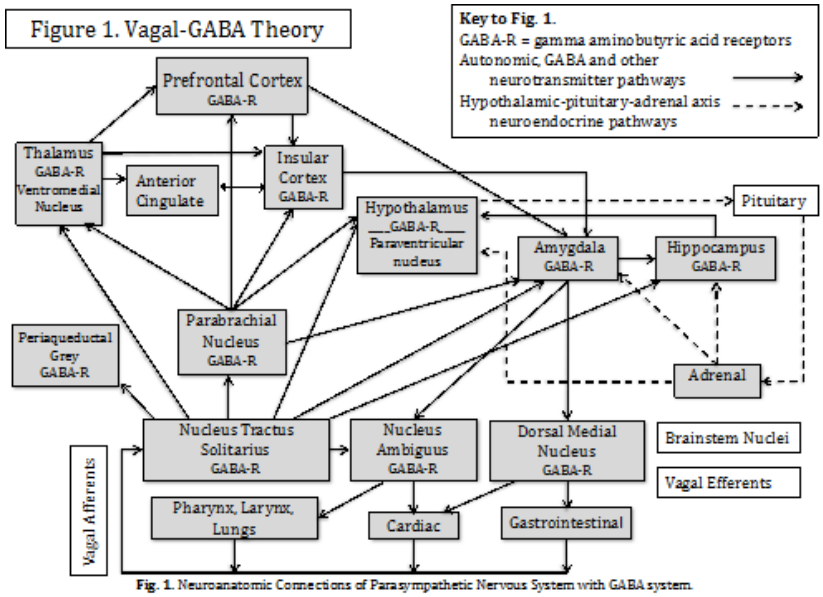

Fig. 1. Neuroanatomic Connections of Parasympathetic Nervous System with GABA system. CC Streeter et al. /Medical Hypothesis 78 (2012) 571-579.

through stretch receptors in the alveolar walls and other respiratory structures (e.g. diaphragm, thoracic musculature), send information to the brain via the vagus nerve. The normal adult respiratory rate is about 12 to 20 breaths per minute. Studies of yoga breathing exercises using slow respiratory rates have demonstrated reduction in sympathetic activity along with increased parasympathetic activity, often measured by increased HF-HRV.(6-8) Vagal afferents in the lungs send information to the nucleus tractus solitarius, the primary input nuclei for the vagus in the medulla; through a series of relays, this leads to the transmission of information through myelinated vagal efferents from the nucleus ambiguous to the sino-atrial node, the cardiac pacemaker (See Figure 1).(9) In healthy controls, Iyengar practitioners, depressed women, and individuals with low back pain, interventions composed primarily of yoga postures are associated with increased HRV. Thus, HRV has become an established biomarker for measuring effects of yoga postures.(10-12) Systematic reviews found risk of bias using the Cochrane Criteria in studies assessing HRV in RCTs comparing yoga with a control intervention.(13) Due to the methodological weakness in the study designs, the review concluded that more research was needed concerning the effect of yoga on HRV. This proposed study fills that gap because it is designed to comply with the Cochrane Collaboration Tool for RCTs.(14) In a study of depressed women assigned to a 12-week Hatha yoga intervention or an untreated control group, there was a significant decrease in Beck Depression Inventory II (BDI-II) scores and a significant increase in HF-HRV.(12) Yoga stretching is considered a form of self-massage, which has been associated with increased HF-HRV and decreased Spielberg State Trait Anxiety Inventory (STAI) scores.(15) The use of RSA, a component of HRV, as a biologic marker for the effect of yoga breathing and postures in this proposal is supported by the above studies that used the BDI-II and STAI with HRV, the same dependent variables used in our dosing study and proposed in this study. Slow breathing exercises have also been linked with improved mood and increased HRV.(16) Subjects with MDD who were treated with biofeedback that used breathing at a resonance frequency of 4.5 to 6.5 breaths per minute, demonstrated increased HRV and improved mood.(8) The results of these studies support the use of RSA as a biomarker for both yoga postures and breathing. The Neurovisceral Integration Model of Thayer (2000) suggests that ANS imbalance, with an underactive PNS, may be the final common pathway between negative emotions and poor health (e.g., depression, PTSD, anxiety, panic), as well as medical conditions (e.g., cardiovascular disease).(17-19) In disorders with stress-induced ANS imbalance, optimizing ANS imbalance entails increasing PNS under activity and/or reducing SNS over activity. Increased HF-HRV, a marker for increased PNS activity, indicates that the stress response system has greater flexibility to respond to challenges.(16) Stress is associated with inflammation and immune imbalance, both of which may be ameliorated by yoga stretching. The connective tissue network is an integrated, whole-body system that is amenable to physical manipulation by massage, yoga, qigong, and tai chi.(20) Fasciae are composed of an extracellular connective tissue matrix that forms structures surrounding every organ of the body, integrates the musculoskeletal system, and houses the blood and lymphatic vasculature.(20) Stiffness and lack of mobility of fascia has implications beyond the inability to move adequately. An extensive literature links physical mobility and health, including mental health.(20) Stretching of connective tissues may have local anti-inflammatory effects independent of vascular, neural, or other systemic factors.(21) Exercises with a prominent stretching component (e.g. yoga) have been found to decrease levels of circulating pro-inflammatory cytokines. In comparison to expert hatha yoga practitioners, the novices' serum IL-6 levels were 41% higher, and the odds of a novice having detectable C-Reactive Protein were 4.75 times as high as that of an expert.(22) In a 6-month RCT, subjects with inflammatory bowel disease (IBD) were randomized to coherent breathing plus qigong movement intervention or an educational control group. The coherent breathing group demonstrated significantly decreased stress, improved mood, reduced IBD symptoms and decreased C-Reactive Protein versus no changes in the control group.(23) That study is the first to show that in patients with IBD (a stress-driven disorder of immune response), coherent breathing and qigong (a Chinese form of yoga) is associated with reduced inflammation (presumably via vagal anti-inflammatory pathways) and deceased stress-related immune imbalances.(23) A meta-analysis of more than 300 empirical articles describes the relationship between psychological stress and parameters of

the immune system in humans.(24) Chronic stress alters immune responses. A change in the immune system from flexible and balanced to inflexible and unbalanced may contribute to increased vulnerability to stress-related immune dysregulation. An additional mechanism whereby yoga postures and coherent breathing alleviate depression may be through counteracting inflammatory abnormalities seen in some depressed subjects.(9)

2.1.c. **Yoga Treatments in Disorders with Low Parasympathetic and GABA Activity:** MDD, PTSD, Obsessive-Compulsive Disorder, Generalized Anxiety Disorder and epilepsy, are associated with low parasympathetic and GABA activity. Interventions that increase parasympathetic activity (measured by HF-HRV or RSA) or increase GABA activity (measured by MRS) are associated with clinical improvements in a number of disorders of clinical relevance to Veterans.(25-36) These disorders demonstrate symptom reduction when treated with pharmacologic agents that increase GABA activity, and when treated with yoga in RCTs.(37-43) Iyengar yoga has been shown to increase parasympathetic tone and has been successfully used to treat depressive symptoms in controlled studies.(11, 44, 45) Further, studies have found yoga to be effective as a monotherapy and as adjunctive therapy for individuals with MDD being treated with antidepressants, but these were not RCTs.(44, 46) Although there is substantial evidence to support the use of yoga in treatment of MDD, a recently published review found yoga to have only Level 3 evidence (Level 1 was highest, Level 4 was lowest), as an adjunctive treatment for depression due to insufficient safety data and non-randomization of studies.(47) Our recently published dosing paper, recently submitted safety paper and the current proposal address these gaps by using the Consolidated Standard of Reporting Trials (COHORT) and the Cochrane Collaboration Tool for assessing risk of bias in randomized trials and by careful monitoring of adverse events for safety data.(14, 48)

2.1.d. **Trauma Response Spectrum:** The Trauma Spectrum Response includes emotional dysfunction, (e.g., depression, anxiety, PTSD and anger), cognitive dysfunction, (e.g., decreased attention, concentration, memory and problem solving), somatic dysfunction, (e.g., fatigue and sleep disturbances), and alcohol and substance use disorders.(49) It is observed that disorders within the Trauma Response Spectrum are co-morbid and share fundamental imbalances – i.e., under activity in the PNS and GABA systems and over activity in the SNS. These observations support the concept that comorbid disorders observed in the Veteran population, such as MDD, PTSD and Alcohol Use Disorder (AUD) are based on the same transdiagnostic biological abnormalities. The use of an intervention that addresses abnormalities in ANS balance is novel, provides a new entry point for treatment and may enhance available treatments. In an RCT of 30 Australian Vietnam veterans, diagnosed as 100% disabled due to PTSD by the Australian VA and who had failed 30 years of treatment trials, including multiple medication combinations, a 5-day multi-component yoga and breathing intervention resulted in significant recovery from PTSD and depression at week-6 compared to the control group, and showed further improvements at 6 months.(50) On the Clinician Administered PTSD Scale, the effect size from baseline to week-6 was high at 0.90 and 2.9 at 6 months. Scores on the Center for Epidemiological Studies Depression Scale (CES-D) and Post-traumatic Stress Disorder Checklist Military (PCLM-17) also decreased significantly from baseline to week-6 and month-6. A similar study in 21 US Veterans of the wars in Iraq and Afghanistan found significant recovery in PTSD, anxiety, and physiologic measures of hyperarousal.(51) The proposed study, by monitoring PTSD symptoms in MDD subjects, will provide information on whether changes in symptoms of MDD and PTSD in response to the proposed intervention are correlated in this population, an observation that would support the transdiagnostic nature of these conditions, and the potential of an intervention that addresses a common and fundamental imbalance, having a far reaching impact on the care of Veterans.(49, 52)

2.2. Preliminary Data

2.2.a. Summary of Work Done: Over the last decade, our group has completed four studies, with

translational and clinical components, demonstrating that yoga interventions are associated with increased thalamic GABA, increased RSA, improved mood, and decreased anxiety. These studies included experienced yoga practitioners, healthy controls, yoga naïve healthy controls, subjects with MDD and low back pain, subjects with MDD and PTSD, and subjects with MDD.(9, 53, 54) Previous studies conducted in civilians were supported by federally funded grants (R21, R01, K03), and resulted in peer reviewed publications, and manuscripts under review and in preparation.

2.2.b. Study 1 (54), Streeter et al 2007, a cross-sectional study, compared changes in GABA levels using magnetic resonance spectroscopy (MRS) in experienced yoga practitioners and healthy controls with no yoga experience at baseline (Scan 1), followed by 60-minutes of hatha yoga practice in yoga practitioners or reading magazines in healthy controls. This was immediately followed by Scan 2. GABA-to-Creatine ratios (GABA/Cr), measured in a 2-cm axial brain slab, showed 27% increase in GABA/Cr in yoga practitioners after the yoga session, but no significant change in healthy controls ($t = -2.99$, $df = 7.87$, $p = 0.018$). This was the first study to document a significant increase in GABA associated with a yoga intervention.

2.2.c. Study 2 (53), Streeter et al 2010 addressed the possibility that increased GABA/Cr in Study 1 could be due to exercise. In a longitudinal RCT, eligible subjects who were yoga naïve, with no Axis 1 disorders, no use of medications known to influence GABA receptors, and no current yoga or mind-body practices received a baseline scan (Scan 1). Subjects were then randomized to a 12-week Iyengar yoga intervention or a group walking intervention that was matched to the yoga intervention for (1) Metabolic Equivalents (METS) by walking 2.5 miles an hour on a flat surface, (2) interaction with research staff and (3) group effect.(55) After the 12-week intervention, subjects underwent Scan 2, immediately followed by a 60-minute yoga or walking intervention, depending on group assignment, prior to Scan 3.

2.2.d. Changes in Mood Scores: Study 2 utilized the Spielberg State Trait Anxiety Inventory (STAI), State and Trait subscales, and the Exercise Induced Feeling Inventory (EIFI) with subscales for Revitalization, Tranquility, Revitalization, and Physical Exhaustion.(56, 57) These scales were selected because they can be given repeatedly to measure current mood states over time. Mood assessments were obtained at baseline, before each scan, and at weeks 4, 8, and 12. Groups did not differ on demographic variables or STAI Trait anxiety scores. Tonic changes over the course of the study (Scan 2 – Scan 1) for mean mood scores showed significant increases in the yoga group for Revitalization (1.8 ± 2.5, $t_{18} = 3.21$, $p = 0.005$). Acute changes from pre- to post-intervention (Scan 3 – Scan 2) for mean mood scores showed significant increases in the yoga group for Revitalization (2.5 ± 2.7, $t_{18} = 4.12$, $p < 0.001$) and Tranquility scores (2.0 ± 1.8, $t_{18} = 4.77$, $p < 0.001$), and a significant decrease in STAI-State anxiety scores, consistent with decreased anxiety (-5.2 ± 5.5, $t_{17} = -4.05$, $p < 0.001$). A Generalized Estimating Equation (GEE) analysis of mean mood scales at baseline, and weeks 4, 8, and 12, showed the yoga group to have significantly greater improvement than the walking group on all subscales of the EIFI, with significant increases in Tranquility, Revitalization and Positive Engagement, and significant decreases in Physical Exhaustion.

2.2.e. Study 3 (9), Streeter et al 2012: This study compared GABA/Cr in subjects with MDD who were enrolled in a 12-week trial of Hatha yoga for low back pain (Saper 2009) and with healthy controls from Study 2.(58) Iyengar yoga is a subset of Hatha yoga. The Iyengar yoga 12-week intervention from Study 2 and the Hatha yoga 12-week intervention for Low Back Pain were reviewed by a certified Iyengar instructor and found comparable. The MRS protocols used to collect GABA/Cr were identical in both studies. This allowed Scan 1, obtained before the 12 week interventions and Scan 2 after the interventions to be compared in healthy controls (n=19) from Study 2 and subjects with MDD and low back pain from Study 3 (n=2). At baseline, the MDD subjects with low back pain demonstrated lower GABA/Cr, which increased over the course of the 12-week yoga intervention, as depressive symptoms and low back pain declined.

2.2.f. Acceptability and Feasibility of Yoga to Veterans (59): A RCT of the yoga for low back pain intervention discussed in C.3.a versus an educational control was conducted in Veterans at the VABHCS, under the direction of Dr. R. Saper, co-investigator of Study 3: 283 Veterans were interested, 210 completed telephone screening, 28 (13%) were ineligible, 182 (87%) met eligibility, 131 (62%) completed in person consent, 120 (57%) were randomized to yoga for low back pain or educational intervention. Randomization was completed in 10 months with follow up for 24 months. Over 90% were male, 49% were Non-Hispanic white. Available data show that out of the 120 randomized subjects, 57 have completed the intervention, 39 are schedule to complete the intervention in March, and 24 are scheduled to complete the intervention in June. These data demonstrate that a yoga protocol similar to the proposed yoga intervention is acceptable to Veterans at the VABHCS, the site of the proposed study.

2.2.g. Study 4 Streeter et, al 2017: Study 4 is a dosing study that filled a gap in the literature, as there were no published studies on the optimal dose of a hatha yoga intervention for MDD. Study 4 refined the Study 2 Iyengar yoga protocol by adding a 20-minute coherent breathing exercise at five breaths per minute to further increase PNS activation, thereby maximizing the effect of the intervention. This increased the intervention to 90-minutes, ~60-minutes of postures, transitions and 20-minutes of coherent breathing. The dosing study was a longitudinal RCT wherein subjects with MDD were randomized to 12 weeks in a high dose group (HDG) of three 90-minute yoga classes per week and four 30-minute homework sessions, or a low dose group (LDG) of two 90-minute yoga classes per week and three 30-minute homeworks. Eligible subjects, 18-64 years old with MDD, had baseline Beck Depression Inventory II (BDI-II) scores ≥14 (with higher scores representing greater depressive symptom severity) and were not on antidepressants, or were on a stable dose of antidepressants for ≥3 months. Exclusion criteria included current psychotherapy for depression, history of psychosis or bipolar disorder, active alcohol or substance abuse, history of suicidal ideation with intent within the last year (suicidal ideation without intent was allowed), current mind-body practices, nicotine use, more than 5 drinks on three occasions in the last 3 months, and medications directly affecting GABA receptors (e.g., sedatives benzodiazepines, antiseizure medications). Anxiety Disorders were allowable and resulted in the enrollment of the following: 8 PTSD current, 1 PTSD in full remission, 4 PTSD sub-threshold, 3 Panic Disorder in partial remission, 1 Panic Disorder in full remission, and 1 Social Phobia current.

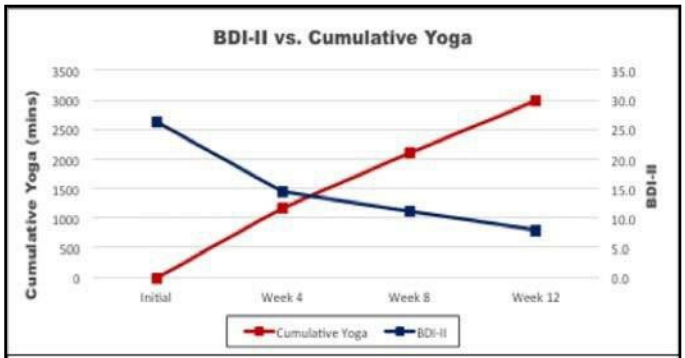

Figure 2: Evidence that Becks Depression Inventory II (BDI-II) scores are significantly negatively associated Cumulative Yoga Minutes, a measure of compliance.

2.2.h. Depressive Symptoms, Compliance and Safety (Figure 2): Retention was excellent, with 93% (30/32) of randomized subjects completing the study: HDG (n = 15), and LDG (n=15). Compliance was excellent for classes, homework and total yoga minutes. Compliance for total yoga minutes was 87 ± 28% for the HDG and 84 ± 19% for the LDG, with no difference in compliance between groups (p = 0.77). This resulted in a significant difference between groups in total yoga minutes with 4,075 ± 1,314 minutes for the HDG and 2,737 ± 625 minutes for the LDG (p = 0.001). There were no serious adverse events related to the study. To our knowledge, this is the first study to report significant yoga dosing differences between groups and demonstrates our group's ability to recruit and retain subjects who are compliant with the proposed yoga protocol. Demographic variables and BDI-II scores at screening did not differ significantly between HDG and LDG. BDI-II scores declined significantly from screening through week 12 for the HDG (-18.6 ± 6.6, p<0.001) and the LDG (-17.7 ± 9.3, p<0.001). As there was no difference between groups in BDI-

II scores, a two-sample paired t-test conducted for the full cohort at baseline (26.2 ± 7.4) compared to week-12 (8.0 ± 6.4), demonstrated a significant reduction in depressive symptoms (t_{29} = -12.5, p < 0.001). A Generalized Estimating Equation (GEE) analysis at baseline, 4, 8, and 12 weeks with BDI-II scores as the dependent variable and Cumulative Yoga Minutes as the independent variable was statistically significant (β = -0.002, z = -2.31, p = 0.02), with depressive symptoms declining as yoga minutes increased (See Figure 2).

2.2.i. Depressive Symptoms and Mood Scales (Table 1): Using a two-sided Fisher's exact test comparison, there were no significant differences observed between groups from baseline through week-12 in either the proportion of responders, > 50% decrease in BDI-II (p=0.65) in 87% of HDG (13/15) and 73% of LDG (11/15), or the proportion of remitters, BDI-II scores ≤14 at week 12 (p = 1.00) in 93% of HDG (14/15) and in 87% of LDG (13/15). Statistically significant differences occurred in the proportion of subjects with BDI-II scores ≤10 at week-12 (p = 0.04), 93% of HDG (14/15) and 53% of LDG (8/15). GEE analysis of mood scales at baseline and weeks 4, 8 and 12 for the full cohort showed significant improvements in mood and decreases in anxiety when corrected for multiple comparisons (Table 1). The Pittsburg Sleep Questionnaire Inventory (n=30) showed significant improvement in sleep from baseline (7.8 ± 3.9) to 12-weeks (5.3 ± 3.6) (t_{29} = -4.91, p < 0.001). Given that the HDG and LDG did not differ for BDI-II scores ≤ 14 at week-12, considering the time demands of a tri-weekly intervention, and the significant results in the Study 2 with two yoga interventions per week, the proposed study will use the LDG model, two yoga interventions per week.

Table 1: GEE Analysis of Mood Scales: baseline, weeks 4, 8, and 12

Instrument	Coefficient	z	p	Clinical Significance
BDI-II	-0.91	-9.66	<0.001	↓ depressive symptoms
STAI	-0.74	-4.49	<0.001	↓ anxiety
EIFI: Positive Engagement	0.1	2.53	0.01	↑ positive engagement
EIFI: Revitalization	0.29	7.85	<0.001	↑ revitalization
EIFI: Tranquility	0.19	5.12	<0.001	↑ tranquility
EIFI: Physical Exhaustion	-0.3	-6.65	<0.001	↓ physical exhaustion

2.2.j. Thalamic GABA/Cr (Figure 3): Figure 3 shows a representative proton spectrum from a LDG subject at baseline (Scan 1), after 12-weeks of yoga and breathing intervention (Scan 2), and immediately after a 90-minute yoga and breathing session at week 12 (Scan 3). There is a noticeable increase in the GABA peak from Scan 1 (blue spectrum) to Scan 2 (yellow spectrum) to Scan 3 (red spectrum). Because the proposed study will use two yoga classes per week, a one-sample t-test comparing the change in GABA/Cr from Scan 1 (pre randomization) to Scan 3 (post 12-week intervention) in the LDG (that also used two classes) shows a statistically significant increase in thalamic GABA/Cr (t_{14} = 2.51, p =0.02).

2.2.k. Reanalysis of Study 2 Data: In Study 2, healthy controls participated in a yoga intervention and study design similar to Study 4, with Scan 1 at baseline, Scan 2 after a 12-week yoga intervention and Scan 3 immediately after a yoga intervention. In both Studies 2 and 4, GABA is reported as GABA/Cr ratios, which is standard in the field of MRS. In order to compare values from Study 2, collected in analog format on the 4T scanner, with values from Study 4, collected in digital format following a system upgrade to the same 4T scanner, Study 2 data were converted to digital format and re-analyzed using the same spectral fitting algorithms as in Study 4. Consistent statistical conclusions were maintained.

2.2.l. <u>Comparison of Thalamic GABA/Cr in Healthy Controls and Subjects with MDD (Figure 4):</u> A fixed effects, omnibus one-way ANOVA, was used to detect differences in GABA/Cr in subjects with MDD (LDG, n=15) from Study 4 and healthy controls (n = 18) from the yoga group in Study 2. This analysis revealed significantly higher GABA/Cr in healthy controls at Scan 1 ($F_{1,32}$ = 6.486, p = 0.016) compared to MDD subjects. Differences between these groups were no longer significant at Scan 2 ($F_{1,32}$=2.636, p = 0.115) or Scan 3 ($F_{1,32}$ = 1.695, p=0.203), as GABA/Cr of subjects with MDD increased (normalized) to the level of non-depressed healthy controls over the 12-week intervention.

2.2.m. <u>Parasympathetic tone measured with Respiratory Sinus Arrhythmia (RSA) (Figure 5)</u>: In Study 4, RSA values were derived from an electrocardiogram (ECG) recording over a three stage protocol: 1) a 5-minute rest, 2) a 3-minute step test that removes the vagal break, and 3) a 5-minute recovery period. RSA was measured at baseline and after an intervention at weeks 4, 8, and 12. No differences between the HDG and LDG were observed for baseline or recovery RSA. A GEE analysis of changes over the 12-week intervention for the full cohort showed no RSA differences at rest (β = -0.01, z = -0.74, p = 0.46), but an increase in RSA during recovery (β = 0.05, z = 2.12, p = 0.03), consistent with the recovery stage being the best marker of tonic changes from the beginning to the end of the study. Midway through the study, an additional RSA was added pre the week-12 intervention, permitting comparison of pre- and post- week-12 intervention RSA values (n=16). Rest stage ($F_{1,15}$ = 11.06, p = 0.005) and recovery stage changes ($F_{1,15}$ = 3.82, p = 0.07) were significant from pre- to post- 12-week intervention. The acute changes in both rest and recovery stages observed from pre- to post-intervention, support the hypothesis that yoga acutely increases resilience measured by increased RSA in the rest and recovery stages. Tonic changes were only significant in the recovery stage, suggesting that the recovery stage is more sensitive to resilience.

2.2.n. <u>Correlation of Thalamic GABA/Cr and RSA:</u> Because the proposed study will use two yoga classes per week, correlational analysis of GABA and RSA was conducted on data from the LDG. Acute changes in GABA were measured at the end of the 12-week intervention from pre-yoga intervention (Scan 2) to post-yoga intervention (Scan 3). At the end of the 12-week yoga intervention, acute RSA changes were also

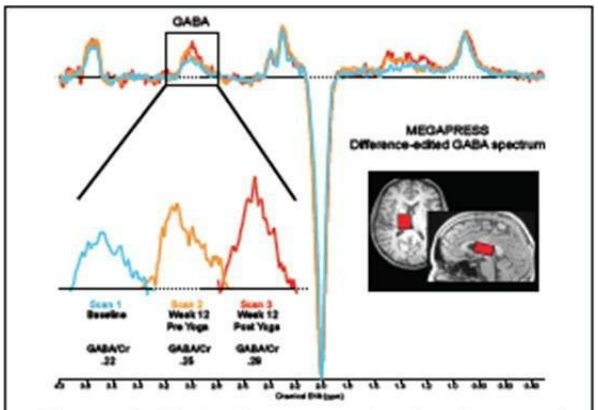

Figure 3: Thalamic spectra showing increased GABA (LDG Subject), Scan 1 pre-randomization to Scan 2 after a 12-week yoga intervention to Scan 3 immediately after a 90-minute yoga and breathing intervention.

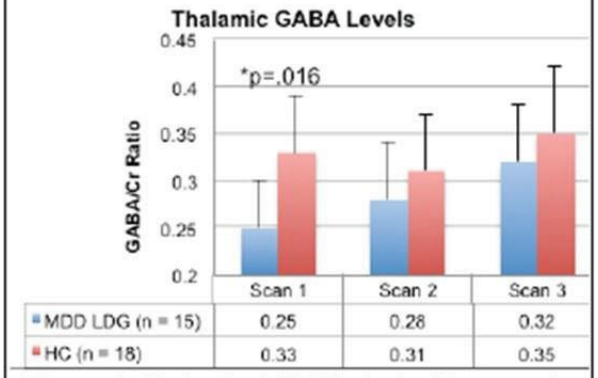

Figure 4: Thalamic GABA/Cr in healthy controls and patients with MDD at baseline (Scan 1), after 12-week yoga intervention (Scan 2) and immediately after a yoga intervention (Scan 3). GABA increases in subjects with MDD, and differences between MDD and controls decreases.

Figure 5: Respiratory Sinus Arrhythmia (RSA) at rest and recovery (after step challenge). Significant changes observed pre-12 week (blue dash, $F_{1,15}$=11.06, p=.005) and post-12 week (red solid, $F_{1,15}$=3.82, p=.07) yoga intervention.

measured before and immediately after a yoga intervention. A Spearman rho correlation (two-tailed) of the difference between this 12 week pre- and post- intervention recovery RSA for Scan 3 – Scan 2 GABA/Cr was statistically significant ($rho_7 = .821$, $p = 0.023$). This supports the hypothesis that RSA is significantly correlated with GABA.

2.2.o. Safety and Reduced Suicidal Ideation under review Streeter: In Study 4, mild side effects related to the study occurred, most commonly muscle soreness (n=13), which resolved over the course of the study. There were no serious Adverse Events related to the study. No subjects developed suicidal ideation during the study; 9/10 subjects reporting suicidal ideation without intent at screening on the C-SSR, with 8/9 reporting the resolution of this symptom over the course of the study. This preliminary evidence suggests that yoga and coherent breathing are safe and effective in the treatment of MDD, and may reduce suicidal ideation.

2.2.p. Yoga as an Adjunctive to Pharmacologic Treatment in Study 3 and 4: Approximately 40% of individuals treated with pharmacology for MDD do not achieve full remission, which places them at increased risk for recurrence and relapse.(3) Study 3 (n = 2) and Study 4 (n = 2) include subjects on stable doses of antidepressants for greater than three months who still met criteria for MDD when enrolled in the 12-week yoga protocol. Subjects were on the following medications that act on the monoamine system: duloxetine and venlafaxine, serotonin-norepinephrine reuptake inhibitors (SNRI); fluoxetine, a serotonin selective reuptake inhibitor (SSRI); and bupropion, a norepinephrine-dopamine reuptake inhibitor (SNDI). All subjects exhibited decreased MDD symptoms with addition of the 12-week yoga intervention, as measured by PHQ-9 in Study 2 and BDI-II in Study 4. These observations support the hypotheses that yoga postures and breathing interventions corrected an imbalance of sympathetic over activity and parasympathetic under activity, at the level of the vagal nuclei in the medulla. The proposed yoga intervention shows promise as augmentation to pharmacotherapy that addresses imbalances in the monoamine systems found at higher levels of the neural axis in the pons and midbrain.

2.2.q. How would the results of this study impact clinical practice and/or plans for future studies? There is evidence that Complementary and Alternative Medicine (CAM) among Veterans may be greater than in the general population, with 50% of the Veterans in the Southern Arizona VA medical Facilities reporting CAM use compared to 36% to 38% of the general US population.(60) In veterans with chronic pain, up to 76% of those not currently using CAM therapies reported wanting to use CAM was if available in their VA facility.(61) A large number of VA facilities provide some type of CAM.(60) There is increasing recognition that multimodal treatments are needed to address issues of depression, anxiety and pain in the VA population. A Mindfulness Based Stress Reduction (MBSR) study in Veterans reported decreased anxiety, depression and suicidal ideation.(60) While this study demonstrates the potential benefit and acceptability of MBSR that included gentle yoga, this was a self-referred open-label study. More RCTs are needed to increase the evidence base such that these types of treatment can be integrated into multimodal treatment plans for Veterans. Suicides in the Veteran population have become an urgent public health concern. Historically lower than civilian suicide rates, in the past decade, suicide rates in the Veteran population have doubled, surpassing death due to being killed in action.(62) The risk of suicide in Veterans with diagnoses of MDD, PTSD, and Alcohol or Substance Use Disorder also is increased. There are few evidenced based therapies addressing suicidality.(63) The proposed intervention has demonstrated effectiveness in reducing suicidal ideation. Over a 12-week trial in MDD, the proposed intervention documented, both response and remission of MDD symptoms, over 80% compliance, mild side effects, and no increases in depressive symptoms.(64) The proposed intervention was effective in reducing depressive symptoms in subjects who were medication free and in subjects who were still depressed on stable pharmacologic treatment for at least 3 months. Therefore, the proposed trial has potential to support a significant vertical change in the effectiveness of care provided to Veterans suffering from MDD, as monotherapy or as an adjunct

to existing treatments. This is not a treatment resistant depression study, broadly defined as failure on trials with at least two pharmacologic agents for an adequate period of time at an adequate dose. This study addresses the need of the 40% of individuals being treated for MDD, who do not achieve asymptomatic remission. The proposed research will employ established Iyengar trained teachers to insure the safety, quality and consistency of the intervention in this efficacy study. Generalizability is increased because the yoga postures in the manualized yoga intervention are used in Hatha Yoga schools and can be provided by certified yoga instructors with diverse training. The stratification of randomization to include current PTSD diagnoses and assessment of PTSD symptoms and alcohol consumption prior to and during the study will provide preliminary data for use of this intervention in PTSD and AUD that have low parasympathetic and GABA activity. This line of research will lay an important foundation for hypothesis testing of other aspects of the Vagal-GABA theory, such as abnormalities in immune and inflammatory systems.

3.0 RESEARCH DESIGN AND METHODS

3.1 Drug/Device Information: NOT APPLICABLE

3.2 Type of Study: (Table 3: Individual Timeline): This is a longitudinal, efficacy randomized controlled trial (RCT). Veterans with MDD will be evaluated for eligibility and randomized to a 12-week intervention of a twice-weekly yoga and coherent breathing or walking group. As there is evidence that exercise improves mood in unipolar depression, the walking group will serve as an active control condition.(47) The policy for Common Data Elements will be incorporated into the data collection matrix. The Consolidated Standard of Reporting Trials (CONSORT) will be used to ensure the quality of reporting for this RCT.(48) The **primary dependent variable** will be changes in the Hamilton Depression Rating Scale (covering a 2 week period), and mood and anxiety scales (designed for repeated use, such that changes from pre to post intervention can be gauged), measured using valid and reliable psychological instruments obtained at screening, intervention weeks 4, 8, and 12, and before each MRS scan. The **secondary dependent variable** will be brain gamma amino-butyric acid (GABA) levels measured by magnetic resonance spectroscopy (MRS) in the left thalamus, the same region used in our prior studies.(9, 53, 65) The thalamus has the technical advantage of providing high quality spectra, has a high concentration of GABAergic neurons, and thus a high GABA signal, in which changes can be detected. The thalamus is also anatomically connected to other regions of interest. GABA levels will be measured by MRS in 3 separate scan acquisitions: before randomization (Scan 1), after 12-weeks of the yoga or walking intervention (Scan 2), followed immediately by the group assigned intervention and the final scan (Scan 3). The **third dependent variable** will be respiratory sinus arrhythmia (RSA) a component of heart rate variability (HRV) that is an accurate index of vagal influences on the heart.RSA will be measured before each scan and before and after an intervention at week 4, week 8 and week 12. Medications also are evaluated at weeks 4, 8, and 12. Two and six month follow-up phone calls will assess depression, quantity of yoga practice, and barriers to practice.

3.3.1. **Sub-Study Participation:** This is not a multisite study. There will be a contract with the McLean Hospital for goods and services regarding the imaging component of this study.

3.3.2. Study Related Procedures

3.3.2.1. **Instruments:** The following reliable and valid instruments will be used during the screening interview to determine eligibility, and to monitor subjects during the interventions:
(1) Structured Clinical Interview for DSM-5 **(SCID-5)** is a widely used valid psychiatric diagnostic instrument used to screen for Axis I disorders(66);
(2) Columbia Suicide Safety Rating Scale (C-SSRS) is a clinically administered instrument to assess suicide risk(67);
(3) Physical Activity Recall (PAR) is a widely used structured interview that yields a weekly record of physical activity using Metabolic Equivalents (METs), a method of assessing an individual's level of physical activity in a common unit that can be calculated from a variety of activities.(55) The PAR will be obtained each week of the intervention in the event physical activity becomes a confounding factor.
(4) Hamilton Rating Scale for Depression (HDRS) is a 17-item clinician administered scale frequently used to evaluate the effectiveness of antidepressants being assessed for FDA approval(68);
(5) Beck Depression Inventory (BDI-II) is a 21-item self-administered instrument designed for the assessment of depressive symptoms, with higher scores reflecting greater severity(69);
(6) Spielberger State-Trait Anxiety Inventory (STAI) is a self-administered instrument designed for assessment of trait anxiety (STAI-Trait) and serial assessments of state anxiety (STAI-State) with higher scores indicating higher anxiety(56);
(7) Clinician Administered Posttraumatic Stress Scale (CAPS) is the gold standard clinician administered assessment for PTSD(70);
(8) PTSD Check List – Civilian (PCL-C) is a 17-item self-administered scale corresponding to Diagnostic and Statistical Manual of Mental Disorders (DSM) IV criteria for PTSD. Because not all Veterans have combat experience and not all trauma is from a military experience, the civilian version of the PCL will be used. The use of the CAPS and PCL-C will allow assessment of severity of PTSD symptoms, and if present, will be modeled in statistical analyses;
(9) Exercise-Induced Feeling Inventory (EIFI) is a self-administered instrument designed to assess four distinct feeling states associated with bouts of physical activity: Revitalization, Tranquility, Positive Engagement, and Physical Exhaustion.(57)

Table 3: Individual Time Line and Collection of Dependent Variables

	Aim 1		Aim2	Aim 3
Individual Time Line	Depression Scales	Mood Scales	GABA	RSA
Telephone Screen				
Screening Session	HDRS/BDI-II 1			
3T Brain Scan				
MRS Imaging Session 1				
Pre Scan 1 Measures	HDRS/BDI-II 2	EIFI & STAI 1		RSA 1
MRS Scan 1(Baseline)			GABA 1	
Randomization				
Week 4				
Pre-Week 4 Evaluation		EIFI & STAI 2		RSA 2
Week 4 Intervention				
Post-Week 4 Evaluation	HDRS/BDI-II 3	EIFI & STAI 3		RSA 3
Week 5-7 Intervention				
Week 8				
Pre-Week 8 Evaluation		EIFI & STAI 4		RSA 4
Week 8 Intervention				
Post-Week 8 Evaluation	HDRS/BDI-II 4	EIFI & STAI 5		RSA 5
Week9-11 Intervention				
Week 12				
Pre-Week 12 Evaluation		EIFI & STAI 6		RSA 6
Week 12 Intervention				
Post-Week 12 Evaluation	HDRS/BDI-II 5	EIFI & STAI 7		RSA 7
MRS Imaging Session 2				
Pre Scan 2 Evaluation	HDRS/BDI-II 6	EIFI & STAI 8		RSA 8
Scan 2			GABA 2	
Interscan Intervention				
Pre-Scan 3 Evaluation		EIFI & STAI 9		RSA 9
Scan 3			GABA 3	
Follow-Up 2 month	HDRS/BDI-II 6	EIFI & STAI 10		
Follow-Up 6 month	HDRS/BDI-II 6	EIFI & STAI 11		

(10) Pittsburgh Sleep Quality Index (PSQI) is a self-administered instrument that assesses 9 factors of sleep over a month period, providing a composite score, with higher scores indicating poorer sleep quality.(71)
(11) Timeline Follow Back (TLFB) will be used to assess alcohol consumption for three months prior to screening.(72) A format similar to the TLFB will be used for nicotine consumption.
(12) Brief Pain Inventory, an eleven point Likert Scale of pain intensity will be used to assess pain over the last 24 hours.
(13) Stroop Color-Word Test is a selective attention and inhibitory cognitive control task.(74)

(14) Digit Symbol Substitution Test (DSST) is one of the frequently used tests to measure psychomotor slowing, and is clinically sensitive because it makes demands on several processes, including perception, working memory, sustained attention and visuomotor co-ordination (Wechsler 1997).

(15) Child Trauma Questionnaire (CTQ) is a self-report measure of childhood trauma. (73) This scale will be given during the Week 4 evaluation at the end of the session.

Unless otherwise stated, instruments 4-12 are used at screening, week 4, 8, and 12. In addition, the EIFI and STAI-State are administered before and after interventions at weeks 4, 8, and 12 and before each scan. The CAPS is administered at screening and at week 12. The following instruments are given at Imaging Session 1 & 2: 2, 5, 13, and 14.

Recruitment Plan and Requirements (Table 4): Veterans will be recruited from the Bedford VA Medical Center (VABHCS) through posters, the internet, providing flyers to clinics, in meetings with staff, and recruitment at clinics affiliated with the VABHCS. Veterans who had a diagnosis of depression in the previous two years at the VABHCS will be identified via electronic health records. Targeted letters signed by the PI describing the study and inviting the Veterans to call or mail for more information will be mailed to Veterans. The mailing will include opt-in or opt-out card with postage and/or an opt-in or opt-out phone number. If a card or phone is not received within 10 days of the mailing, the identified Veterans will be called concerning their interest in the study. Data Access Request Tracker (DART) and VA Informatics and Computing Infrastructure (VINCI) will be used to identify potential subjects.

3.3.2.2. If interested they will be provided information about the study and will be allowed to proceed to the telephone interview. These techniques have been used successfully in previous VABHCSstudies of yoga interventions.(59) The VABHCS Institutional Review Board (IRB) will pre- approve all forms of advertisement. Current VA policy does not allow email communications as part of the recruitment procedures. Sample size calculations, based on power analyses, require the following number of completers equally divided between the yoga and walking groups to test the hypotheses for each dependent variable: **n=70** completers to test Aim 1: Depressive Symptoms and Mood Scale; **n=56**to test Aim 2: GABA changes; and **n=68** to test Aim 3: RSA changes [D.20]. To meet the criteria for each Aim/Dependent Variable n=70 subjects will complete the intervention with n=56 having usable MRS data. Using data from Study 4, with consideration of changes in inclusion and exclusion criteria and research setting, the following recruitment schedule will be required during a 5-year study period, which will include over 4 years of recruitment and treatment. The required total recruitment and annual requirements at each stage are as follows: telephone screen=420 total or 105/year; screening interview=173 total or 43/year; randomization=100 total or 25/year; and completers=68 total or 18/year.

3.3.2.3. **Participation in Non-Assigned Group: A Retention Plan:** Prior to randomization, subjects will be asked their group of choice (i.e., yoga or walking), as not being assigned to a group of choice could influence dropout rate. In order to retain subjects not assigned to their choice, subjects will be allowed to participate in the group they are not assigned to for 16 sessions or 8 weeks (i.e., the second intervention), whichever comes first, if they complete their randomized intervention and 14 week follow up phone call. Retention in the randomized intervention is given priority over contamination of the 6-month follow up with the 2nd intervention. There will be no difference in how subjects in the randomized intervention or second intervention will be monitored during the first eight weeks of the intervention. Subjects will not be paid for class attendance or evaluations during the 2nd intervention nor will they undergo MRS scanning. These data are exploratory.

3.3.2.4. **Screening:** Subjects will be screened by telephone to prevent subjects from coming to the screening interview, only to be excluded based on information that could be obtained over the phone. Telephone questions do not include questions about self-harm or risk of danger. Telephone questions do include questions about metal and implanted medical devices that could preclude MRI scanning. If subjects meet telephone-screening criteria, they will be invited for an in-person screening interview at

the VABHCS. Subjects will be provided with the written consent to review prior to the screening interview. Written informed consent approved by the ENRM VA IRB will be obtained by research staff, defined as being able to assess participants using the Hamilton Depression Rating Scale(HDRS), the Clinician Administered Posttraumatic Stress Scale (CAPS) and assessing subjects for safety issues using the Columbia Suicide Severity Rating Scale (C-SSRS). All subjects will be consented by the PI who will conduct a medical history, physical exam and MRI safety screening prior to scanning. After the informed consent is signed, but before data collection, subjects will take a quiz to assess their knowledge of the study and undergo a breathalyzer test to determine Breath Alcohol Concentrations (BrAC). If BrAC is not zero or the subject fails to demonstrate knowledge of the study onthe quiz, subjects will not be allowed to participate in study activities. If the subject answers a quiz question incorrectly, that question will be discussed with the subject, after which the subject will initial the correction. If the study clinician determines the subject does not understand the protocol, including risks of non-conventional therapy, this would constitute reason for exclusion. The PI will review the inclusion/exclusion criteria to determine eligibility prior to the subject being invited to participate in the study.

Potential participants are excluded for a history of psychosis, bipolar illness, and suicidal ideation with intent or a suicide attempt within the last year. Axis-I disorders that would interfere with study participation are exclusionary such as Obsessive Compulsive Disorder or Agoraphobia that would prevent attendance at the intervention. Alcohol Use Disorders with more than 3 DSM-IV criteria. Substance Use Disorders in Remission are allowed. Because inversion can increase intraocular pressure, individuals with a retinal hemorrhage or glaucoma cannot participate unless they receive clearance from their ophthalmologist. Neurologic conditions, which would effect scan results, are exclusionary such as stroke, seizures, brain surgery, HIV and other CNS disorders. The ability to participate in both interventions is required, such that a person who walks with a cane would not be enrolled as it would be a concern that they would either not be able to walk at 2.5 miles an hour for one hour or that if they did it could potentially harm them. Individuals with a history of spine surgery could be excluded depending on the surgery. Individual with cardiovascular or respiratory disease would require careful evaluation that could include a discussion with their physician. Traumatic Brain Injury with greater than 30 minutes loss of consciousness would be exclusionary. During the screening process, the potential participants have to complete a 3-minute step test (See Step Protocol). If the participant is unable to perform the step test, they would be excluded from the study. If during the physical exam, the PI is concerned about the potential participants ability to participate, they maybe asked to attempt to touch their toes, and to get down and then up from a yoga map or to walk the circuit that they would walk during the walking intervention.

3.3.2.5. Inclusion Criteria:

(1) male and female Veterans ages 18 to 65;
(2) Fluent in English;
(3) Understands the risks and benefits of the study as listed in the Post Consent Quiz
(4) Females must agree to use an acceptable form of birth control [see 4.6.1.15 for acceptable contraception] and do not intend tobecome pregnant during the study
(5) Females have a negative pregnancy test or a serum progesterone in the non luteal stage defined by less than or equal to 1.3 ng/ml prior to Scan 1.
(6) Meets criteria for MDD on the SCID-5
(7) Hamilton Rating Scale for Depression (HDRS) scores ≥ to 14 at screening
(8) If subjects have been taking antidepressants that target a monoamine system, the dose has been stable for at least one month with no anticipated changes during the study; or no antidepressants for one month
(9) If subjects have been in a stable form of psychotherapy for three months, with no anticipated changes in their psychotherapy during the study (this would exclude time-limited manual-driven therapies such Cognitive Behavioral Therapy); or no psychotherapy for three months
(10) Reliable contact information provided
(11) Subject weighs up to 300 lbs. at the discretion of the PI.

(12) Subject has completed all required screening instruments and evaluations

3.3.2.6. Exclusion Criteria:

(13) History of psychosis (substance-induced psychotic disorder and psychotic disorder due to another medical condition are allowable if fully resolved)
(14) History of bipolar illness
(15) History of suicidal ideation with intent in the last year according to the Columbia Suicide Safety Rating Scale (C-SSRS)
(16) Subject has a history of suicidal attempt with intent to injure in the last year according to the C-SSRS.
(17) Desire to be treated for MDD with a new treatment during the study such as pharmacotherapy somatic therapy or psychotherapy.
(18) A contraindication to magnetic resonance evaluation (e.g., pregnancy, a cardiac pacemaker, ferrous implant including shrapnel, or intrauterine devices (IUDs) with copper, claustrophobia that would prevent scanning, some tattoos with black ink on the head including permanent eyeliner).
(19) Current mind-body practice (e.g., yoga, Tai Chi, Qigong, breathing practices, or meditation) defined as more than 6 one-hour sessions in the last 6 months.
(20) The use of intra-uterine devices with progesterone that interferes with the ability to determine the non-luteal phase required to schedule Imaging Session 1.
(21) Participates in physical exercise > 5 hours/week that is equivalent to or greater than 6 metabolic equivalents (METs) in intensity.
(22) Has been treated psychotropic medications such as mood stabilizers (e.g., Valproic Acid, Carbamazepine, or Lithium) in the last three months.
(23) Subject has been treated a) with a scheduled dose of anti-anxiety agents (e.g., benzodiazepines) or sleeping aids (e.g. Ambien or sedative hypnotics) or b) with pain medication other than Non-Steroidal Anti-Inflammatory Drugs (NSAIDS) in the last one month except for procedure related pain management (e.g., dental procedures).
(24) Has greater than three current criteria for Alcohol or Substance Use Disorder using DSM-V criteria.
(25) Does not have a period of 48 hours of no alcohol use on TLFB to participate in scanning given the effects of alcohol consumption on the GABA.
(26) Has a neurologic condition, such as a Traumatic Brain Injury (TBI) with a loss of consciousness of greater than 30 min, that in the opinion of the PI could compromise subject safety or the integrity of the study.
(27) Has a medical condition that in the opinion of the PI could compromise subject safety or the integrity of the study.
(28) Subject would not be expected to complete the study including scheduling issues.
(29) Has an Axis-I diagnosis, other than depression except as listed, that in the opinion of the PI would interfere with the subject's participation in this study.
(31) Has participated in another research study in the last 3 months that could affect the dependent variables.
(32) Must have a period of 24 hours of no nicotine use on TLFB to participate in scanning or nicotine use as allowed by CSD&R Project Manager.
(33) Subject does not agree to study procedures designed to protect against COVID-19 and the requirements of the virtual interventions.

3.3.2.7. **Randomization:** Group assignments will be conducted using permuted block randomization with varying block sizes. Block sizes will be multiples of the number of treatments and take the allocation ratio into account. For this study with a 1:1 randomization of 2 groups (yoga and walking), block sizes will be 2, 4, and 6. Randomization will be computer generated with each randomization assignment put on a card in numerically labeled envelopes that will be sealed. The envelopes will be opened in sequential order at the time of randomization by a member of the staff that is not blinded to group assignment. Subjects who want more time to think about participation can be randomized within 12 days of the last screening assessment, with the interventions starting within 7 days.

3.3.2.8. **Group Assignment, & Blinding:** All investigators are blind to group assignment prior to randomization. Blinding will be guided by the Cochrane Collaboration Tool for assessing risk of bias in randomized trials.(14) The study physicist, Dr Zuo, and Dr.Heilman, the RSA expert, will be blind to group assignment. Investigators who administer subjective instruments (i.e., Hamilton Rating Scale for Depression and CAPS) will be blind to group assignment. Subjects will fill out self-reporting mood and psychological scales in paper form that will be entered into the VA network by research staff. All VA paper documents with sensitive information will be secured in a locked filing cabinet in a designed VA Office. ECG data used for RSA analysis and MRS data will be labeled with a blinding code provided by the Data Coordinating Center, such that ECG data and MRS data are stripped of identifying information, including removal of study ID, date of acquisition and timing of the assessment (baseline, pre or post the intervention at weeks 4, 8, or 12) before being sent Dr. Heilman for analysis. As subjects are not blind to the intervention, there is no procedure to break the blind for clinical reasons.

3.3.2.9. **Gender Differences:** While we will equally recruit both genders, due to the demographics of the VABHCS population, it is anticipated that more males will be recruited, which will providemuch needed information on the efficacy of this intervention in men. Due to the influence of menstrualcycle phase and associated changes of progesterone on brain GABA/Cr levels, serum levels of progesterone will be drawn before each scan, with progesterone levels less than or equal to 1.3 ng/ml being considered the non-luteal stage, a stage when GABA/Cr is not influenced by menstrual cycle.(75) Serum progesterone levels will be drawn at the VABHCS.

3.3.2.10. **Subject Compliance and Payment:** Compliance with group session attendance and homework is important, both as an assessment of treatment acceptance and as a measure of the "dose" of the intervention required for symptom reduction. Attendance at the interventions will be recorded on an attendance sheet; home practice of yoga and walking will be recorded on the weekly PAR. Subjects will be paid for their time: $10 for each group session, $20 for the week 4 evaluations, $40 for the week 8 evaluations, $60 for the week 12 evaluations, and $40 for each MRS scan for a maximum of $480.

3.3.2.11. **Yoga Intervention**: The Iyengar method uses props such that the postures can be modified to meet the physical abilities of the subjects. The yoga manual developed in Study 2 and 4 emphasizes backbends and chest opening postures consistent with the recommendations of Iyengar method for depression.(76) All yoga classes in the proposed intervention will be conducted according to the manual. Each class follows the progression of seated/reclining poses, Sun Salutation standing poses, spinal twists, back bends, forward bends and inversions and a deep relaxation (savasana). Consistent with their training, the yoga instructors are allowed to adjust poses and sequences to meet the needs of the subjects. The 90-minute yoga sessions include approximately 60-minutes of yoga postures, 10-minutes of transition and deep relaxation and a 20-minute CD of paced coherent breathing practice at a rate of 5 breaths per minute. In the Iyengar method, breathing practices start with exercises that use equal periods of inhalation and exhalation.(77) Coherence breathing uses 4 to 6 soft breathes per minute with equal periods of inhalation and exhalation consistent with the Iyengar method. This proposed study uses coherence breathing at 5 breaths per minute, an optimal rate for most people and a method successfully piloted in Study 4. Subjects will be instructed to attend 2 classes per week for a total of 24 classes. Missed classes can be made up as long as the total dose of 24 classes is not exceeded. Homework sessions for the yoga group are 15-minutes of yoga postures and 15-minutes of coherent breathing. Subjects will be provided with a yoga mat, 2 yoga blocks, 1 yoga belt, and three yoga blankets and a manual of the yoga sequences, and a CD of the paced breathing for at home practice. Additional props maybe provided for use during the virtual intervention as determined by the yoga instructors. The B.K.S. Iyengar Yoga National Association of the United States (IYNAUS) Certification Committee certifies teachers, in the B.K.S. Iyengar method for the practice and teaching of yoga. Ms. Elizabeth Owens, head yoga teacher for Study 2 and 4, will recruit and manage the teaching schedule

for certified yoga instructors with Iyengar training who have at least 5 years teaching experience. Instruction in coherent breathing is necessary because the positive effects on sympatho-vagal balance depend not only on the respiratory rate, but also on degree of effort, depth, and muscle relaxation while breathing. Without proper instruction, some inhale too rapidly, overfill their lungs, exhale too forcefully, overuse abdominal muscles, or breathe paradoxically (contracting abdominal muscles while inhaling). Any of these errors could increase sympathetic activation, interfere with parasympathetic activation or even cause hyperventilation. (16) Instructors are needed to observe subject breathing and to monitor and provide corrective feedback.

The COVID-19 pandemic has resulted in all of the yoga instructors providing yoga classes virtually. In order to support social distancing the yoga interventions will be provided virtually through a platform approved by the ISSO officer, such as VA Video Connect. The yoga instructors will teach all yoga interventions virtually. The subjects will be required to attend the randomization, and weeks 4, 8, and 12 interventions at the VABHCS so that equipment can be provided or collect and pre and post intervention data can be collected.

3.3.2.12. **Walking Intervention:** The walking intervention was developed and used in Study 2. The literature documents the benefit of physical activity in the treatment of depression, such that a walking group can be considered an active control.(47) For the in-person walking intervention, subjects walk in an indoor circuit with a defined length such that the number of circuits required for a rate of 2.5 miles an hour rate are completed in 60- minutes. Subjects are monitored so that they complete 25% of the required circuits every 15-minutes. The time for the group walking intervention is 60-minutes per group session and the homework is for 15-minutes three times a week, beyond typical walking. The walking group and homework are designed to match the physical activity of the yoga group (i.e., time spent doing yoga postures), not the length of yoga sessions, this results in the walking intervention taking less time than that of the yoga group, with the extra time in the yoga group due to the breathing exercise that does not include group interactions or physical exertion. Study staff will be trained to monitor the intervention and direct subjects to ensure the correct number of laps are completed.
In order for the interventions to be as balanced as possible, the subjects assigned to the interventions will need to come to the VABHCS the same number of times as the subject assigned to the yoga intervention. For the virtual walking sessions, subjects will check in with research staff at the beginning and end of each session by video or telephone. Virtual walking sessions will also include walking at 2.5 miles an hour on a flat surface. During the screening process, subjects will be evaluated for stride length,such that the number of steps to equal 2.5 miles will be calculated. At the randomization session, subjects will be instructed on how to use the pedometer. Subjects will wear a pedometer for each walking session, both in-person and at home, the number of steps for each session will be recorded four times during the intervention and at the end of the homework to document the distance walked. Subjects can provide documentation of the number of steps by photographing the pedomenter and sending an email or text or by reporting to the walking intervention monitor during the session.

3.3.2.12.1. **Yoga and Walking Fidelity Assessment and Schedules:** The Yoga and Walking Fidelity Assessments have 10 components that will be monitored for fidelity to the protocol. Each yoga instructor, and each walking intervention monitor will have an assessment for each quarter of study participation. An evaluation that has less than 100% fidelity to these 10 components will trigger a review of the evaluation, and a follow up evaluation will be completed within four weeks. The fidelity assessment can be used for in-person and virtual interventions. Four group sessions will be offered each week for the yoga and the walking intervention. The yoga and walking groups will be held at the same time to prevent blinded staff from deducing group assignment based on the time of the assessments. The PI may change the intervention schedule or number of session to meet recruitment and retention needs.

3.3.2.13. **Clinically Significant Abnormalities:** If a participant's tests have clinically significant

abnormalities or unexpected findings, they will be told. Specifically, females will be told if they are pregnant. If the serum progesterone level is not less than or equal to 0.03 ng/ml, the participant will be told and possibly asked to repeat the test. If there is a clinically significant finding on the anatomic MRI, the participant will be informed by the study PI, an M.D., with in 7 days of receipt of the report by phone or in person. It is McLean's policy to call the listed physician if there is an abnormality is found during the MRI reading that requires immediate attention. If the participant gives written permission a copy of the report will be forwarded to the identified physician.

3.3.2.14. **Assessment for Suicidality and Safety Plan:** Participants are screened weekly for suicidality by use of an Adverse Events sheet that they are required to turn in at the first session of the week, either in-person or virtual. This form may be collected by the Project Manager, or research staff. Due to the addition of virtual interventions, the form can be filed out by research staff over the phone. If there is an indication of increased depression of suicidal ideation, the project manager will inform the research staff or the PI that an assessment of the participant is required. There are also in-person evaluations by research staff at weeks 4, 8, and 12 were participants are assessed for suicidality and increased depressive symptoms. This study excludes individuals with a history of suicidal ideation with intent or a suicide attempt in the last year. This study allows suicidality without intent, which is some individuals is present for years.
Assessment of suicide risk is done using the Columbia Suicide Severity Rating Scale (CSSR-S). The development of suicidality with intent based on the CSSR assessment will result in study withdrawal such that the participant can receive additional conventional treatment. If a member of the research staff concludes that suicidality with intent is present they will develop a safety plan with the participant that can include support by friends or family, the inclusion of their medical treaters, being taken to the VABHCS Mental Health Clinic, where admission to an in patient unit can be arranged. If there is a safety concern after 3:30 when the Mental Health Clinic closes, participants will be referred to Urgent Care or the Lahey Clinic that provides after hours emergency services for the VABHCS. If the participant can be managed safely as an outpatient, arrangement will be made for follow-up treatment. It is noteworthy suicidality decrease in Phase 1 in civilians and no subject were with drawn from the study for increased symptoms.

3.3.2.15. **Potential Problems**: Recruitment goals will be assessed every 6 months. If recruitment falls behind schedule, study staff will meet with the staff from the numerous clinic programs at the VABHCS to inform them of this research opportunity. If enrollment in the imaging component is behind due to nicotine use causing more than 10% of exclusions in a 6 month period, the inclusion criteria regarding smoking will be increased to include all subjects, regardless of abstinence.

3.3.2.16. **Acquisition of Mood Scales and Psychological Tests:** Participants will enter self-report mood and psychological scales onto paper forms. Investigator completed psychological mood and psychological scales will be entered into the REDCap database by research staff. The computers ued for storage of mood scales and psychological tests will be VA computers.

3.3.2.17. **Acquisition and Analysis of GABA MRS and MRI images**: Acquisition and Analysis of GABA MRS and MRI images: All MRS/MRI data will be collected on a Siemens 3T scanner at the McLean Imaging Center (MIC), McLean Hospital, Belmont, MA. High-resolution, 3D T1-weighted anatomical images will be acquired to prescribe the voxel with anatomical precision and reproducibility. Placement algorithms developed in our prior studies will be used for consistent voxel placement, based on anatomic landmarks, in the left thalamus (2x2x3 cm). A single-voxel proton MRS GABA difference-editing technique, MEscher-GArwood Point-Resolved Echo Spectroscopy Sequence – MEGAPRESS, will be used for spectral acquisition, as it permits detection of high-quality GABA spectral data. MEGAPRESS allows for collection of TE 68ms PRESS spectra for the detection of other important metabolites such as creatine, NAA, choline and glutamate. MEGAPRESS is a well-established GABA spectral difference-editing method that isolates the GABA doublet at 3.00 ppm in vivo.(78) When used in conjunction with PRESS (point-resolved spectroscopy). Resting state data

has been collected at pre-randomization in Study 4, and now can be collected pre and post the intervention to assess for changes in resting state over the course of the study. A resting-state fMRI scan of 8-10 minutes will complete the one-hour scanning. If time constraints require, the resting-state fMRI scan will not be done. Minor adjustments in the protocol within the safety parameters that do not change the science of the study are allowed.

As discussed with VACO, if there is a problem acquiring scans at the McLean Hospital (e.g. system upgrade) the study can proceed without the imaging component without a protocol deviation.

3.3.2.18. **Acquisition and Analysis of PNS Measures:** This protocol was used in Study 4. The skin will be cleaned with an alcohol wipe prior to placement of three electrodes at heart level in the left mid-axillary line, above the umbilicus, and under the right clavicle. ECG data will be collected using a GGP Biolog IBI monitor (UFI, Morro Bay, CA) in three consecutive phases: (1) resting, while sitting quietly for 5-minutes; (2) step, a 3-minute challenge using the YMCA Step protocol; and (3) recovery, a 5-minute sitting period. The YMCA Step Test is a standardized test used for 30 years as part of the YMCA Fitness Testing and Assessment Manual.(81) The Official YMCA test consists of stepping up and down on a 12-inch step in time to a 96 per second metronome for a total of 26 step cycles per minute. As in Study 4, the test will be modified to use an 8-inch step to increase subject ease. The step challenge is designed to remove the vagal brake such that RSA deceases and then increases during the recovery period.(82) ECG data will be obtained prior to randomization, and pre- and post-intervention at week 4, week 8, and week 12, and before each MRS scan. The ECG data will be acquired using a Faros Bittum 180 and software approved and installed on VA computers htransferred to a VA computer using the 3991xGPP Biolog progam (see attached Faros Bittum manual). The data will be blinded and sent to Dr. Heilman for analysis.

3.4. Data Collection

3.4.1. Discussion of VA research activities and data for non-VA research activities and non-VA data: All activities conducted under this study are for research proposes. The VABHCS is the coordinating center through which all recruitment, advertisements, participant assessments, and interventions associated with this research project will occur with the following exceptions. There will be a contract with the McLean Hospital for the acquisition, and analysis of the MRI data. There will be an contract with Dr. Keri Heilman for the analysis of the ECG data. The contracts and agreements will clearly state that the data acquired and analyzed are good and services and belong to the VA.

3.4.2. Data collection, storage, access, use, disclosure, and analysis:

3.4.2.1. Describe all data collection activities for VA research:
The collection of imaging data at the McLean Hospital was approved by VACO. There are three dependent variables: 1) Hamilton Depression Rating Scales, 2) Thalamic GABA levels, and 3) Respiratory Sinus Arrhythmia (RSA).

3.4.2.1. Psychological Scales: All data from screening and intervention assessments will take place at the VABHCS and will be put into REDCap. Evaluations at the McLean Hospital will be put into REDCap using a VA issued laptop.

3.4.2.2. Spectroscopic Data: All magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) data used for analysis of GABA levels will be collected and analyzed at the McLean Hospital under a contract that will state that the data collected under the contract belongs to the VABHCS. The analyzed data will be transferred via secure electronic means for incorporation in the final data set. All individuals scanned at the McLean Hospital are checked in using their name, address and social security number.

3.4.2.3. Formal Reading of MRI Study: It is the policy of the McLean Hospital that all research subjects have a VA brain scanning sequence that is formally read by a person qualified to read MRI studies. This formal reading does have the subject name on it. The data that will be used for the study will only be marked with the subject ID.

3.4.2.4. RSA Data: Electroencephalogram (ECG) data will be collected 9 times during the study, three times at the McLean Hospital prior to each scan and before and after evaluations at weeks 4, 8, and 12 at the ENRMH. This data will be collected using a hand held device, a Bittum Faros, and transferred to a VA laptop computer where it will be processed and blinded before being sent through secure electronic means to Dr. Keri Heilman, Co-Investigator for analysis. Dr. Heilman will then sent the analyzed data back for incorporation into the final database. The software that will be used to transfer the data from the Bittum Faros will be put on the VA computers by the VABHCS Information Technology (IT) staff.

3.4.2.5. Storage: Data will be stored in the REDCap database that will be created within the VA system, scan data will be stored in the locked McLean hard drives which as backed up regularly, the original ECG recordings will be stored on the VA network in a designated file. The ECG data sent to Dr. Heilman will not contain HIPPA identifiers, when the data is returned it will be saved on a VA computer. The final data set will incorporate REDCap, imaging and RSA data. The management of the REDCap database and merging of the other databases into the final data will be done by the study statistician and Healthcare Specialist in accordance with VA regulations. The desktop computer will be stored in the Project Manager's Office, The laptops will be stored in Dr. Streeter's and the research staff's office and will be taken to the McLean Hospital as needed. Dr. Streeter will also be Teleworking such that she will take her computer with her to her home and appointments, this computer will be encrypted. Papers with personal information will be kept in the offices of Dr. Streeter, the Project Manager and research staff in locked filing cabinets when not in use. Data regarding scan acquisition will be kept in a locked filing cabinet at the McLean Hospital or on secure servers. A Final Copy of the Database will be stored at the VABHCS on the VA network. One copy will be stored on the PI's VA laptop, a third copy will be provided to the study statistician. The database is owned by the VA.

3.4.2.6. Access and Use: Only member of the research team and individuals providing goods and services through contracts or Interagency Personnel Agreements (IPAs) will have access to the data. When study personnel are no longer members of the research team, they will no longer have access to the study data. The exception would be through the Data Management Access Plan

submitted with the Merit application.

3.4.2.7. Statistical Analysis: The data from REDCap, MRI/MRS and RSA will be merged in to a final dataset for analysis by study statistician. The final dataset will be a Limited Data set in which the only HIPPA identifier will be the date of data collection. This data collection and analysis plan was included in the submitted proposal that was approved by the VACO, such that they were aware that data was going to be acquired and transferred outside of the VABHCS.

3.4.2.8. Security Measures: There are security measures in place at the VABHCS, the Data Coordinating Center, and the McLean Hospital.

3.4.3.1. Will VA data be combined with non-VA data: Not Applicable, all data is VA data, as data collected at the McLean Hospital is obtained under a contract. VACO has approved for Dr. Streeter to be off-site at the McLean Hospital to oversee the imaging component of the study. Dr. Streeter has appointments at the VABHCS, the McLean Hospital, and Boston University School of Medicine, the site of the Data Coordinating Center. VACO has approved Dr. Streeter to have off-site time, such that she can use combined data while on VA time.

3.4.3.1. Identify any VA research activities occurring at non-VA sites: All imaging acquisition and analysis will take place at the McLean Hospital. Dr. Heilman will conduct analysis of the ECG s for RSA off site with data that does not contain personal identifiers but will contain the data of acquisition. The study statistician will merge the data from REDCap, analyzed spectroscopic data, and RSA data into a final dataset.

3.4.3.2. Existing Protocols: Not Applicable

3.4.3.3. Provide a copy of any memorandum of understanding (MOU) with the non-VA entity describing data ownership or data security arrangements for the "collaborative" study: The McLean Contract provided for IRB approval and contains information regarding data ownership and data security. The contract for data management and data security that will contain Memorandum of Understanding (MOU) or contract describing the data ownership and security arrangements will be submitted to the IRB for approval before the data management and analysis can proceed.

3.4.3.4. This protocol does not collect non-VA research.

3.4.3.5. Uses and Disclosures of Protected Health Information (PHI): The data will only be used for research. The REDCap, ECG and MRI databases will only identify participants by their subject ID. Of the 18 HIPPA identifiers, only the date of date collection will be included in the Limited Final Database. The Final Database will include the dates of the assessment, which would not pose a reasonable risk of allowing the subject to be identified. There will be a separate database in REDCap with contact information, such that the PI or other research staff can contact subjects. The protections relating to HIPPA will be followed.

3.4.3.6. Researchers and Study Team Members: All research team members will be identified in the IRB approved study. Study team members are defined as non-VA employees who are involved in the contracts. Only researchers and study team members will have access to the data with the exception of the required regulators. While at the McLean Hospital, member of the McLean staff, who are not part of the research team, will interact with subjects at check in.

3.4.3.7. The Principal Investigator, Dr. Chris Streeter will have at the start of the study the following appointments: 1) a 3/5th appointment at the VABHCS VA; 2) an appointment at the Boston University School of Medicine for which she is compensated for work that is non-overlapping with this study; and 3) a without compensation appointment at the McLean Hospital.

(i) VA duties: The PI's 3/5th VA appointment includes clinical time as a member of the department of psychiatry at VABHCS and her duties as the PI of this study

(ii) VA duty locations: The VA duty locations are the VABHCS for for clinical duties related to VABHCS and the conduct of this study.

(iii) VA tours of duty or time allocations: The PI' 3/5th VA appointment is equivalent to 24 hours a week plus a 30 minute meal break for period of work over 4 hours. This study will be run using the same structure that has worked for the civilian version of this study. In order to provide intervention times such that individual who are and are not employed can participate in the study, the interventions will be conducted from 8 am to 9:30 am and from 5 pm to 6:30 pm on Monday and Wednesday, the days of the intervention can be changed or additional sessions added as needed. The week 4, 8 and 12

evaluations that take place before and after the interventions and take around 45 minutes. To accommodate this schedule, Dr. Streeter's VA tour of duty will be from 7:30 am to 8:00 pm on Monday and Wednesday which includes a 30 minute meal break. After subtracting 30 minutes for lunch each day this results in two 12-hour tours, 24 hours or 3/5th time.

(iv) Issues related to data ownership: All data collected at the McLean Hospital will be collected under a contract which will state that the data belong to the VABHCS. All data analyzed by Dr. Heilman belongs to the VABHCS. Research information protection and data security requirements. The protocol approved by Veterans Administration Central Office (VACO) included permission for Dr. Streeter to be off site to oversee the collection and analysis of data at the McLean Hospital and for Dr. Heilman, Dr. Zuo, the study physicist to have access to a Limited Data Set. HIPPA identifiers will remain within the REDCap database, the VA Medical Health Record and the McLean Medical Health Record.

3.5. Analysis Plan

3.5.1. Data Management and Analysis Plan: The study statistician and Healthcare Specialist will be responsible under a contract for data management and statistical analysis for the project.The Healthcare team will convert the REDCap shell from an earlier study and provide the monitoring reports, create of the final database. The study statistician will oversee the statistical analysis with the help of the Healthcare Specialist. Descriptive statistics will be generated for baseline data and distributions between the yoga intervention and walking intervention will be compared using two-sample t-tests and chi-square or Fisher's exact tests toassess effectiveness of randomization. Descriptive statistics include means and standard deviations forcontinuous variables and group sizes and proportions for categorical variables. All analyses with be performed at the $\alpha = 0.05$ level of significance. Should groups have unequal distributions at baseline, relevant variables will be incorporated in subsequent linear models that will be used to examine intervention efficacy. In order to address the study aims of assessing the relative longitudinal change independent variables between yoga intervention and walking intervention groups over time, data will be collected at four time points, pre-randomization, and at weeks 4, 8, and 12. Mixed linear model analyses will be preformed focusing on the group x time interaction for the primary effects to be statistically modeled. These analyses will allow for inclusion of data at differing numbers of time points per subject if needed. We will examine different within-subject correlation structures for our interaction model and determine the optimal structure by comparing Akaike information criteria (AIC) values. The correlation structure that yields the lowest AIC will be adopted. Subjects who change antidepressant medications during the study will be included in intent to treat analysis, but will be co-varied for medication changes. Potential cohorts effects will be statistically addressed by controlling for group profiles, including group size, breakdown by sex of attendees, instructor, group parameters such as individual or group sessions, and group cohesion/overlap.

3.5.1.**Data Managment:** The study statistician will provide leadership and expertise in data collection, management and analyses, encompassing database design, implementation of quality control procedures, technical support, and data sharing. A close interaction between data management staff and research team members will result in procedural consistency, efficiency of the study-wide systems, and ultimately produce the highest quality data. Data collection strategies will include web-based assessments using VA REDCap software. When possible, data will be collected on desktop computers, or laptops, negating the need for double entry. Electronic forms will include required fields, skip patterns, and validation resulting in more complete and "cleaner" data at capture. All participants will be assigned a unique identifier (ID) and personal identifiers that must be collected and used for consent and follow up will be stored in a separate database apart from study data. Analysed data from McLean Hospital and Dr. Heilman will be provided via a VA approved system (e.g., AZURE)

3.5.1. Analysis of Hypotheses (Aim 1: Hypotheses I. Aim 2: Hypotheses 2. Aim 3:Hypothesis 3)

Hypothesis I. a. Depressive Symptoms: The yoga group will have decreased depressive symptoms compared to the walking group. There will be a significant within-group change (decrease) in

depressive symptoms over time, using a mixed linear model analogue of repeated measures analysis-of-variance with independent groups in MDD subjects assigned to the yoga group.

Hypothesis 1. b. Mood Scales: There will be a greater improvement in mood (as measured by the EIFI and STAI), and sleep (as measured by the PSQI) in the yoga group compared to the walking group.

Hypothesis 2. a. GABA: GABA levels will increase in the yoga group. There will be a within-group change in GABA levels over time using a mixed linear model analogue of repeated measures analysis-of-variance with independent groups in MDD subjects assigned to the yoga group.

Hypothesis 2. b. Group Differences: There will be a between-group difference in GABA levels over time using linear modeling comparing the yoga group with the walking group.

Hypothesis 3. a. RSA: There will be tonic increase in post intervention recovery RSA in the yoga but not the walking group.

Hypothesis 3. b.: There will be an increase (from baseline to end of week 12) in the difference between pre- and post-intervention in the rest and the recovery RSA in the yoga group.

Hypothesis 4. a. Exploratory Concurrent Antidepressants: The yoga group but not the walking group will have a decrease in depressive symptoms regardless of antidepressant treatment at the beginning of the study.

Hypothesis 4. b. Exploratory PTSD Symptoms: If PTSD symptoms are present at screening, there will be a greater decrease in PTSD symptoms in the yoga compared to the walking group as measured by CAPS and PCL-C.

Hypothesis 4. c. Resting State Connectivity will increase over the course of the study with greater increases in the yoga group.

Hypothesis 4. d. Executive Function will increase over the study as measured by the Stroop, Digit Symbol test and fMRI activation.

3.5.2. Power Analysis for Depressive symptoms and Mood Scales: Based on the power analyses below, in order to achieve adequate power for all analyses, n=70 subjects, n=35 in the yoga and n=35 in the walking intervention must complete the interventions. Using Study 4 data in subjects with MDD in the LDG, the repeated measures ANOVA for EIFI scores from screening to the completion of the intervention did not reach statistical significance in the group of n=15 (baseline: 3.1 ± 2.2 and week-12: 3.9 ± 2.7, $F_{1,13} = 1.097$, $p = 0.31$), a post hoc power analysis was conducted, which determined that the achieved effect size was f =.281, with 52% power. Based on this same effect size, an a priori power calculation was conducted, which determined that **n=35** subjects would be needed to detect significant differences for BDI-II, STAI, and EIFI scores from screening to the completion of the intervention, with effect size f =.281, with < =.02 to account for multiple comparisons, and 81% power. As the power analysis for the EIFI requires the largest number of subjects (n=70), which will be used to determine sample size. In order for 70 subjects to complete the intervention, assuming a 20% drop out, 84 subejcts will be randomized.

3.5.3. Power Analysis for GABA: Consistent with our analytic plan for the longitudinal questions at-hand, we can compute the statistical power for Hypothesis II an analysis sample **of n=26** subjects in each of the study groups using the mixed linear model analogue of repeated measures analysis-of-

variance with independent groups. We used nQuery Advisor, version 7.0 for these computations. Using GABA level as the dependent variable of interest and employing the observation from Study 2 in which the walking intervention showed no change in GABA over the 12-week intervention, it is predicted that GABA levels will not change over time in the walking group. We hypothesize that the yoga group will show mean GABA/Cr = 0.28, 0.285, and 0.32 at the three study time points and that the walking group will show mean GABA/Cr = 0.28, 0.28, and 0.28. Assuming a common standard deviation at each time point of 0.06 and a within-subject correlation of 0.65, we will have 80% power to detect the above noted pattern of repeated mean GABA/Cr with a two-sided alpha of 0.05. With the same data configuration, we will have 85% power should the within-subject correlation be as high as 0.70.

3.5.4. Tonic changes in Recovery RSA from screening to 12 weeks: The GEE of changes in recovery RSA in the full cohort (n=27) over 12 weeks was significant in Study 4; however, only the recovery RSA values in the smaller LDG (n=14) were used for the power analysis, as this reflects the dosage that will be utilized in the proposed study. While the repeated measures ANOVA did not reach statistical significance in the LDG (screening mean = 7.7 ± 2.5 and 12 week mean = 8.2 ± 2.9, $F_{1,12}$ = 1.094, p = 0.315), a post hoc power analysis was conducted, which determined that the achieved effect size was f = 0.291, with 52% power. Based on this same small to medium effect size, an a priori power calculation that includes two groups (yoga and walking) was conducted, which determined that **n=34** subjects would be necessary to detect significant differences in RSA at effect size f = 0.291, with 90% power.

3.5.5. Acute changes in RSA at 12 weeks (pre and post yoga intervention): Half way through Study 4, an RSA collection was added prior to the 12-week yoga intervention, which allowed acute changes in RSA to be calculated. An ANOVA of rest stage changes reached statistical significance; 12-week pre intervention resting mean = 4.9 ± 18 and 12-week post intervention resting mean = 5.7 ± 1.6 ($F_{1,14}$ = 11.056, p = .005). A post hoc power analysis was conducted, which determined that the achieved effect size was f =.858, with 99% power. Based on this same large effect size, an a priori power calculation was conducted, which determined that **n=10** subjects would detect significant differences at effect size f =.858, with alpha = .01 and two groups (yoga and walking) would achieve 90% power. An ANOVA of rest stage changes reached statistical significance; 12 week pre intervention recovery mean = 6.7 ± 2.4 and 12 week post intervention recovery mean = 8.1 ± 3.4 (F = 3.819, p= 0.070). A post hoc power analysis was conducted, which determined that the achieved effect size was f = 0.504, with 96% power. Based on this same large effect size, an a priori power calculation was conducted, which determined that **n=20** subjects would detect significant differences at effect size f = 0.504, with α = .01 and two groups (yoga and walking) would achieve 90% power.

4.0. HUMAN SUBJECTS

4.1. General Characteristics: Subjects will be 18 to 65 year old Veterans with a diagnosis of Major Depressive Disorder. Anxiety Disorders, including Post Traumatic Stress Disorder, and Alcohol or Substance Abuse Disorder with three or less criteria met according to Diagnostic and Statistical Manual of Mental Disorders 5 (DSM-5) criteria will be allowed as long as these disorders will not interfere with the subject's safe participation in the study or interfere with the integrity of the study. While there are more males than females being served by the hospital, more women than men chose to do yoga, such that the percentage of female participants maybe greater than the percentage of females served by the hospital.

4.2. Inclusion of Vulnerable Subjects and Special Populations: Children are included as 18 to 21 years olds. Enlistment in the Armed Services requires an individual is at least 17 years old with parental consent. As enrollment, discharge and registration at the VABHCS for care would take at least a year, such that this study of Veterans cannot include subjects under 18 years old. This study will recruit and include males, females and minorities. The Targeted Enrollment Table is based on the following estimates: Females 10%, Hispanics 6% and African Americans 11%. This study includes

individuals with mental illness; however, the inclusion/exclusion criteria are such that these individual would have the capacity to understand the risks and benefits of the study such that they could make an informed decision. This study does not enroll pregnant females as confirmed by a pregnancy test and the requirement to use an acceptable form of birth control. Due to the possibility of therapeutic misperception by the participants as to what is research and what is clinical care, it is clearly stated that this is a research study that does not overlap with clinical care and the participants can withdraw at any time with no effect on their clinical care.

4.3. Inclusion of Incompetent Subjects: Individuals without capacity will not be enrolled in this study. This study does enrolling individuals with psychiatric illness who will based on the inclusion and exclusion criteria have the capacity to consent.

4.4 Inclusion/Exclusion Criteria

4.4.1. Inclusion Criteria:

1 male and female Veterans ages 18 to 65;
2 Fluent in English;
3 Understands the risks and benefits of the study as listed in the Post Consent Quiz
4 Females must agree to use an acceptable form of birth control [see Human Subjects] and do not intend tobecome pregnant during the study
5 Females have a negative pregnancy test or a serum progesterone in the non luteal stage defined by less than or equal to 1.3 ng/ml prior to Scan 1.
6 Meets criteria for MDD on the SCID-5
7 Hamilton Rating Scale for Depression (HDRS) scores ≥ to 14 at screening
8 If subjects have been taking antidepressants that target a monoamine system, the dose has been stable for at least one month with no anticipated changes during the study; or no antidepressants for one month
9 If subjects have been in a stable form of psychotherapy for one month, with no anticipated changes in their psychotherapy during the study (this would exclude time-limited manual-driven therapies such Cognitive Behavioral Therapy); or no psychotherapy for 1 month
10 Reliable contact information provided
11 Subject weighs up to 300 lbs. at the discretion of the PI.
12 Subject has completed all required screening instruments and evaluations

Exclusion Criteria:

13 History of psychosis (substance-induced psychotic disorder and psychotic disorder due to another medical condition are allowable if fully resolved)
14 History of bipolar illness
15 History of suicidal ideation with intent in the last year according to the Columbia Suicide Safety Rating Scale (C-SSRS)
16 Subject has a history of suicidal attempt with intent to injure in the last year according to the C- SSRS.
17 Desire to be treated for MDD with a new treatment during the study such as pharmacotherapy somatic therapy or psychotherapy.
18 A contraindication to magnetic resonance evaluation (e.g., pregnancy, a cardiac pacemaker, ferrous implant including shrapnel, or intrauterine devices (IUDs) with copper, claustrophobia that would prevent scanning, some tattoos with black ink on the head including permanent eyeliner).
19 Current mind-body practice (e.g., yoga, Tai Chi, Qigong, breathing practices, or meditation) defined as more than 3 one-hour sessions in the last 3 months.
20 The use of intra-uterine devices with progesterone that interferes with the ability to determine the non-luteal phase required to schedule Imaging Session 1
21 Participates in physical exercise > 5 hours/week that is equivalent to or greaterthan 6 metabolic equivalents (METs) in intensity
22 Has been treated psychotropic medications such as mood stabilizers (e.g., Valproic Acid,

Carbamazepine, or Lithium) in the last three months.

23 Subject has been treated a) with a scheduled dose of anti-anxiety agents (e.g., benzodiazepines) or sleeping aids (e.g. Ambien or sedative hypnotics) or b) with pain medication other than Non-Steroidal Anti-Inflammatory Drugs (NSAIDS) in the last one month except for procedure related pain management (e.g., dental procedures).

24 Has greater than or equal three current criteria for Alcohol or Substance Use Disorder using DSM-V criteria

25 Must have a period of 48 hours of no alcohol use on TLFB to participate in scanning given the effects of alcohol consumption on the GABA.

26 Has a neurologic condition, such as a Traumatic Brain Injury (TBI) with a loss of consciousness of greater than 30 min, that in the opinion of the PI could compromise subject safety or the integrity of the study.

27 Subject with a medical condition would not be expected to complete the study, or their participation would be jeopardized subject safety or the integrity of the study

28 Subject would not be expected to complete the study including scheduling issues.

29 Anxiety disorders with current symptoms that would impair participation in the study (e.g., Obsessive Compulsive Disorder (OCD) or agoraphobia that would prevent intervention attendance, Post Traumatic Stress Disorder with dissociation or flashback that could be triggered by the interventions

30 Has an Axis-I diagnosis, other than depression except as listed, that in the opinion of the PI would interfere with the subject's participation in this study

31 Must have a period of 48 hours of no alcohol use on TLFB to participate in scanning given the effects of alcohol consumption on the GABA.

32 Has participated in another research study in the last 3 months that could affect the dependent variables

33 Subject does not agree to study procedures designed to protect against COVID-19 and the requirements of the virtual interventions.

- **Race, Gender, Ethnicity and HIV Status**

Potential participants will not be excluded based on their race, religion, or ethnicity. HIV positive status will results in exclusion due to the potential effect on the imaging data and thus the integrity of the study. Exclusion due to HIV would be made under exclusion number 20**:** has a neurologic or medical condition that in the opinion of the PI could compromise subject safety or the integrity of the study. There is no exclusion related to gender. However a transgender individual taking hormones would result in exclusion due the inability to determine the non-luteal phase of the menstrual cycle such that the first imaging session could be scheduled.

- **Fairness of Recruitment**

Recruitment will be done by flyers, contacting individuals who have the diagnosis of depression, meetings with unit teams and staff. All interested subjects will be screened and included or excluded using the stated criteria.

4.5. Recruitment

4.5.1.1. **Subject Identification and Pre-Enrollment Screening:** Subjects will be identified through flyers, advertisements, interactions with VA staff and through the electronic database. Potential subjects who have not called asking for information will first be contacted by mail which will include instructions on how subjects can opt in or opt out by calling a phone number. The telephone interview form shows the information that will be collected and does not include a social security number. An opt out approach is requested such that subjects can be contacted and given the opportunity to ask questions if they are undecided on whether they wish to participate.

4.5.1.2. **Consent for Recruitment and/or Screening:** Subjects will be read the telephone informed consent over the phone, or if they request a copy will be mailed to them. Subjects are provided with a copy of the written informed consent by mail if requested or are given the opportunity to read the written informed consent or prior to the screening interview. To avoid coercion, subjects are told that they can withdraw at any time without jeopardy to their medical care. To avoid therapeutic misconception, subjects are told that this is a research study that it is not part of clinical care. A request for a waiver of documentation of informed consent is requested for the telephone informed consent. Participants will sign, date and put the time of the consent on the informed consent at the beginning of the screening interview prior to the collection of any data, after the consent has been reviewed by research staff and subjects have had the opportunity to ask questions. The research staff, defined as being trained to use the Hamilton Depression Rating Scale (HDRS), the Clinician Administered Posttraumatic Stress Scale (CAPS) and assessing subjects for safety issues using the Columbia Suicide Severity Rating Scale (C-SSRS) will obtain the informed consent, and will also sign, date and put the time on the consent on the informed consent at the time of the informed consent. The study physician may obtain the initial informed consent, if not he/she will sign the informed consent when the subject is consented with regards to the medical/imaging aspect of the study. There is a clinical reporting form titled Enrollment Progress note that is filled out at the time of the consent. After the consent, subject take a quiz to determine if they understand the risks, benefits and procedures related to the study. This process is conducted in a private room to ensure subject privacy. As subjects have the capacity to consent, there will be no use of surrogates. The subject will be given a copy of the signed informed consent.

4.5.2. Enrollment

4.5.2.1. **HIPPA Authorization:** Enrolled subjects will sign a HIPAA authorization form to provide the following information : Information from VA Health Records such as diagnoses, progress notes, medications, lab or radiology findings; Specific information concerning: alcohol abuse, drug abuse, HIV; Demographic Information such as name, age, race; Questionnaire, Survey, and/or Subject Diary; Imaging Data, electrocardiogram data, physical exam, blood and urine tests.

4.5.2.2. Waiver of HIPAA Authorization: Waiver of the HIPAA authorization requirement is required for recruitment purposes only. HIPAA authorization will be sought from participants prior to enrollment. The information requested is for screening and recruitment to achieve enrollment. We will also conduct brief screenings with veterans determine eligibility. The research uses the telephone screening information to identify subjects to be invited for an in-person screening interview. Recruitment also requires the assistance of DART, and VINCI that are approved in the protocol to further identify subjects. Data regarding recruitment will be obtained from DART, CARRI/JVL, CAPRI/JVL/Remote Medical Record/CPRS and VINCI, with national access to the medical record/EHR through CAPRI. The telephone interview form is designed such that all identifiable information is listed on the first page. All medical and study information is listed on the subsequent pages that are identified by subject ID. At the end of the interview, the first page is separated from the reset of the documents. All personal identifiable information is kept in a separate file from information identified by the subject ID. JVL and CPRS may be accessed by the study PI to determine eligibility. Recruitment: Data will be transferred from VINCI to local server by using VINCI download tool to the Custodians at the VA Bedford Healthcare system. All research staff have VA issued computers and VA emails. The data will not be taken off the VA computers except to print mailing labels for recruitment. Data will not be taken outside of the VA firewall.

4.5.2.3. **Informed Consent:** There are two stages in the informed consent process. The first is consent for a telephone screening for which a waiver of written informed consent was granted. Potential subjects are read an approved consent form that contains all the components of a written informed consent. The member of the VA research team that obtains the telephone informed consent will sign and date the telephone informed consent form. After the participant agrees to participate in

the telephone screening they are asked the questions on the telephone screening questionnaire by any member of the Research Staff. If the participant pasts the telephone screening, they will be invited for an in-person screening interview and/or given the option of being consented with docu-sign that this study has been approved to use. The advantage of docu-sign allows much of the screening procedures to be conducted over the phone or VVC, such that social distancing between potential subjects and research staff is decreased. The initial written informed or docu-sign consent maybe obtained by a research staff, defined as an individual able to use the Hamilton Depression Rating Scale (HDRS), theClinician Administered Posttraumatic Stress Scale (CAPS) and assessing subjects for safety issues using the Columbia Suicide Severity Rating Scale (C-SSRS). Both the participant and the research staff will sign, date and place the time of the signature on the written informed consent or using docu-sign. This will allow the screening interview to proceed. Prior to scanning, a physician (e.g., the study PI), will complete theinformed consent process with the subject with regards to the medical and scanning aspects of the study at which point they will sign, date and place the time of the signature on the written informed consent. The written informed consent or docu-sign is obtained in a private office such that the participant can ask questions. In all instances, the participant would have to have an in-person evaluation for a physical exam and for the informed consent regarding imaging to be signed by the study PI prior to advancing to an imaging session. During the telephone screening for which a waiver of written informed consent is requestedthe subjects may give

consent to be contracted by AZURE so that they can be provided with a copy ofthe written informed consent to review before the in person interview.

4.5.2.4. **Definition of Enrollment and Randomization:** Participants are considered enrolled in the study when they give oral consent to the telephone interview. There is no requirement for written consent to the telephone interview. Participants are considered enrolled in screening after they sign

the written informed consent. Participants are considered randomized when they participate in their first intervention. All subjects who consent to the telephone interview are given a subject ID number and are listed on the master database that links the subject name to their subject ID. The telephone interview has two parts. The first page the telephone interview contains identifying information; this page is separated from the interview after the subject is assigned a subject ID that is used to mark the telephone questionnaire. The information in the telephone informed consent does not collect information regarding drug abuse, alcohol abuse, HIV or sickle cell.

4.5.2.5. **Recruitment Procedures:** Subjects will be screened by telephone to prevent subjects from coming to the screening interview, only to be excluded based on information that could be obtained over the phone. Subjects can be provided a copy of the telephone consent to read prior to the telephone screen if requested. If not they will be read the telephone informed consent over the phone. A waiver of written consent is requested for the telephone screen as it covers questions that could be asked during a routine medical appointment and does not include questions about self-harm or risk of danger. The guide for the telephone interview is provided as a Clinical Report Form (CRF), this guide consist of a page with identifying information such as telephone number, email and address so that information can be sent to the subject. On a different form identified by a subject ID is the text that guides the interview and the information that will be collected. The request for a waiver of written consent for the telephone interview was granted for Phase 1 and Phase 2 of the civilian study on which this study is based. In previous studies, approximately 1 out of 3 subjects advances from the telephone interview to the screening interview. The telephone interview prevents potential participants from having to come for an interview when the telephone screen is guided by a form that does not include social security numbers. Telephone contacts will be done on subjects who respond to advertisements or letters. If subjects meet telephone-screening criteria, they will be invited for an in-person screening interview at the VABHCS. Subjects will be provided with the written consent to review prior to the screening interview. Written informed consent approved by the VABHCS IRB will be obtained by research All subjects will be consented by the PI, a M.D., who will conduct a medical history, physical exam and MRI safety screening prior to scanning. There are two staff signature lines on the informed consent, as the research staff can perform the screening interview except for the procedures that must be performed by an M.D. All consent procedures will take place in a room that will ensure the participants privacy. Advertisements or letters being sent to potential participants will be submitted to the IRB for approval prior to use.

4.5.2.6. **Creation of a VHA Health Record:** This study enrolls outpatients who are receiving a treatment intervention that may lead to physical or psychological adverse events and will be using the phlebotomy lab to collect blood for serum progesterone levels and pregnancy tests, which will be identified by the subjects VA identifiers. Because this study will request a Certificate of Confidentiality, a VHA health record will not be created. Instead a note entry will indicate that an individual has been enrolled in a research study, any details that would affect the subject's clinical care, and the name and contact information for the investigator conducting the study. Subject's informed consent forms and HIPPA authorization documents are not to be included in the health record. No pharmacologic agents are being given as part of the study, no research results being used for clinical care, the study interventions include a yoga or walking intervention, psychological forms and assessment instruments, electrocardiogram data, and imaging studies at the McLean Hospital.

4.5.2.7. **Creation of a McLean Health Record:** The McLean Hospital will create a medical record for the anatomic scan in which the subject will be identified by their name, date of birth, address, telephone number and social security number. This is a McLean Hospital policy and they will obtain the required HIPPA authorization for this record. This record will be kept separate from the subject's research information in which they will be identified by their study

identification number.

4.5.2.8. **Enrollment:** Participants are considered enrolled in the study when they give oral consent to the telephone interview. The Project Manager and research staff will usually do the telephone-

interview consent and telephone interview. Participants are considered enrolled in screening after they sign the written informed consent. The written informed consent can be obtained by research staff, however, the study MD needs to consent the subject with regards to the risks of the imaging part of the study prior to scanning. There are two lines on the IC for staff, such that the research staff can start the evaluation without the study M.D. Participants are considered randomized when they participate in their first intervention. This study uses walking as an active control. There is considerable literature to support the use of exercise as a treatment for depression, such that there is no deception in this study.

4.5.2.9. **Recruitment**: This proposal contains a randomized controlled trial in a clinical population in which 70 subjects will complete the protocol and around 420 will have a telephone-screening interview. Although this efficacy trial is not large or multi-centered, it is considered a Phase 2 trial due to the previous trial in healthy controls (Study 2) and the dosing trial in depressed subjects (Study 4).

Sample size calculations, based on power analyses, require the following number of completers equally divided between the yoga and walking groups to test the hypotheses for each dependent variable: n=70 completers to test Aim 1: Depressive Symptoms and Mood Scale, n=56 to test Aim 2: GABA changes and n=68 to test Aim 3: RSA changes. To meet the criteria for each Aim/Dependent Variable 70 subjects will complete the intervention with 56 having usable MRS data. Using data from Study 4, with consideration of changes in inclusion and exclusion criteria and research setting, the following recruitment schedule will be required during a 5 year study period, which will include over 4 years of recruitment and treatment. The required total recruitment and annual requirements at each stage are as follows: telephone screen 420 total or 105/year; screening interview 173 total or 43/year; randomization 100 total or 25/year; and completers 70 total or 18/year over a 4 year recruitment period.

4.6 Risk/Benefit Ratio

4.6.1. Potential Risks and Methods to be Used to Minimize Risks

4.6.1.1. **Source Materials:** All data collected in this study will be used specifically for research purposes. Types of data will include scores from psychological instruments, screening and monitoring forms, electrocardiogram (ECG) data and imaging data. To ensure confidentiality, all data collected will be marked with subject IDs not the subjects name, this includes paper and electronic collection. An exception to this will be serum progesterone and B-HCG levels that will be processed via the VABHCS laboratory. A master sheet with individual names and their respective code numbers will be kept in a separate locked file or password-protected computer file that can only be accessed by the research staff. The information gathered will only be used for scientific, educational, or instructional purposes. Only the research team will have access to the records. Exceptions to this rule will be made for representatives of the FDA, other U.S. government agencies, and other mandatory bodies as required by law. Forms or requests with subject names include 1) the identifiable information clinical reporting form (CRF), and 2) signed Informed Consent. These forms and any other form with subject names will be kept in a file separate from the other CRFs.

4.6.1.2. **Major Depressive Disorder (MDD) Risks:** There are a number of controlled treatment studies where the practice of yoga and exercise has been associated with decreased depressive symptoms. Neither the practice of yoga or exercise in the form of walking has been established as treatments for MDD. Subjects may experience increased depressive symptoms during the study. There maybe yoga or walking program outside of this study available in the VA or in the community

4.6.1.3. Magnetic Resonance Imaging (MRI) Risks: Claustrophobia while inside the magnet for the magnetic resonance (MR) assessments can potentially precipitate a panic attack in some participants. MR procedures do not involve ionizing radiation and therefore pose no significant radiation risk to participants.

However, risks do exist for persons who have metallic objects near vital structures, such as pacemakers or

aneurysm clips. Individuals with shrapnel from bombs or iron filings in the eye (mostly welders) would be at risk as well. We have a detailed MR Safety Screening Form that every person must complete before he/she receives an MR scan. The greatest risk associated with a MR exam is during the time the participant is escorted into the magnet suite or away from the magnet, as nearly all of the adverse events in MR suites have involved a ferrous metal projectile being drawn into the bore and striking the person. Our sites have very extensive safety awareness plans in place complete with detailed signage of magnetic field strength. All personnel who escort participants or patients into the scanner receive mandatory training and certification. In addition, the imaging sites have complete code carts equipped with cardiac monitors, defibrillators, suction, oxygen, and a full range of medications for any medical emergency that might occur.

4.6.1.4. **Psychological Risks:** Subjects may find questions about their history distressing or uncomfortable. They may feel nervous when answering psychological questions. A member of the research team will be with the participant at all times during the study sessions and will be able to answer questions concerning procedures and psychological tests. Subjects will be informed (by trained study personnel and the informed consent form) that they can decline to answer any questions, decline any procedures, or withdraw from the study at any time.

4.6.1.5. **Alcohol Related Risks:** As subjects will be allowed to have an Alcohol Use Disorder with up to three criteria using DSM V criteria, there is a possibility that they would come to the intervention intoxicated and could injury themselves due to intoxication.

4.6.1.6. **Physical Risks:** The inclusion/exclusion criteria are designed to exclude subjects who would be at risk from participation in the yoga or walking interventions. Subjects may experience muscle stiffness or discomfort from the physical components of the interventions. In Study 2, healthy yoga naïve controls were randomized to a yoga or walking intervention. In Study 4, yoga naïve subjects with MDD were randomized to two or thee yoga sessions a week. Adverse events were monitored on a weekly basis during the 12-week intervention. In Study 2, minimal physical discomfort related to the interventions was reported in both the yoga and the walking group, with slightly greater discomfort reported in the yoga group. In Study 4, subjects reported muscle soreness associated with the yoga intervention (13/30 subjects) that resolved with modifications of the postures. There is minimal risk of injury during the recording of the electrocardiogram (ECG) data using the Faros 180 Sensor (Bittium USA, Inc, Bothell, WA), a hand held device powered by a 3,7 volt battery. There is some discomfort associated with the placement and removal of the electrodes. Some subjects, primarily males due to chest hair, find the removal of the ECG electrodes to be uncomfortable.

The YMCA Step Test is a standardized test that has been used for 30 years as part of the YMCA Fitness Testing and Assessment Manual. For this study a modified version of the YMCA step test (Step Test) is used as the 12-inch step has been reduced to an 8-inch step to make the procedure easier for subjects with short stature. In this protocol, the test consists of stepping up and down an 8-inch step in time to a 96 per second metronome. There are four beats to each cycle (step up foot 1, step up foot 2, step down foot 1, step down foot 2) for a total of 26 step cycles per minute. Subjects will do the YMCA Step Test for three minutes with a 6-minute rest before and after. The YMCA Step test will be done 7 times during the protocol. Subjects could find the Step-Test to be physically challenging or difficult to preform safely.

4.6.1.7. **Confidentiality Risks:** The yoga and walking interventions are conducted in groups. While subjects are informed that what occurs in the group is confidential, there is no guarantee that a participant may say something outside of the group that does not respect the confidentiality of the group. While source documents are marked with a subject code, there is a risk of a loss of confidentiality. Participant could be seen coming to the sessions and a person could deduce that the participant is depressed. There is a risk that the electronic data will not be secure.

4.6.1.8. **Data Transfer Between Collaborating Sites:** All procedures involving subjects will take place at the VABHCS. Data management and statistical analysis will be done through a contract with the Boston University School of Public Health Biostatistics, Epidemiology, Data Analytics Center and or by the VABHCS staff a Bedford VA statistician, both have worked on other VA sponsored research projects and are familiar with VA procedures regarding the handling of data. VA regulations regarding the transfer of data will be followed.

4.6.1.8.1 **COVID-19 Risk**: The COVID-19 pandemic presents novel risk of infection to both the subjects and research staff. A detailed response to this risk has been approved by the VABHCS IRB.

PROTECTION AGAINST RISKS

4.6.1.9. **Recruitment and Informed Consent Risk Protection:** Documentation will be provided to the VABHCS that the study personnel have up to date and the appropriate training in the following areas; Collaborative Institutional Training Initiative (CITI) for the ethical principals of human research, training in Good Clinical Practices (GCP), Privacy, Cybersecurity, and VA Research Data Security and Privacy. The research team will also receive training about the HIPAA regulations. This study will include children in the 18 to 21-year-old range who will be able to understand and complete the informed consent used for all subjects. The consent process will be done in a private space to insure participant confidentiality. During the consent process, all subjects will be informed that this is a research study and the use of yoga or walking for the treatment of Major Depressive Disorder (MDD) are not a conventional forms of treatment. Subjects will be informed that conventional treatments for MDD are available, such as psychotherapy, pharmacotherapy, and somatic therapies (e.g., electroconvulsive therapy, transcranial magnetic stimulation). Subjects are informed that they can change their mind about wanting to have conventional treatment at any time and that the treatment of their depression is more important than the completion of the study. Treatment options will be discussed if subjects decide not to participate in the study. The Principal Investigator's primary clinical responsibilities are at the VABHCS. In order to avoid confusion between what is treatment and what is clinical care, the informed consent will address the issue of therapeutic misconception suchthat the difference between clinical care and research will be clearly defined and the subjects will be informed that if they decline to participate in the study, there will be no effect on the clinical care that they receive. REDCap, a secure web application for building and managing online surveys and databases, will be used to manage data.

4.6.1.10. **Risk of Depression:** This is a treatment study with modalities that are not approved for the treatment of depression. There are alternatives to participation, such as pharmacotherapy, psychotherapy or electroconvulsive, and subjects will be informed of these other options and of their right to withdraw from the study to seek conventional treatment. Subjects are screened for risk of suicide during the screening interview with the Columbia Suicide Safety Rating (C-SSR), a widely used instrument to assess suicide risk. It is noted that the C-SSR does not provide a score stating that the assessment of suicide is a clinical decision. For this reason, other than the development of suicidal ideation with intent, the decision to withdraw a subject remains a clinical matter that is informed by the assessments built into the study. There are assessments by a psychiatrist or member of the research staff licensed to assess for suicide risk at screening, and at the weeks 4, 8, and 12 assessments using the C-SSR. Safety Data from Study 4 showed that out of 30 subjects, 9 out of 10 subjects that endorsed suicidal ideation without intent at screening no longer endorsed suicidal ideation without intent at the end of the study. No subject developed suicidal ideation with or without intent during the study. Suicidal ideation with intent with in a year of screening was exclusionary. In addition, the C-SSR evaluation were in agreement with reports of self harm on the Beck's Depression Inventory II (BDI-II), consistent with both clinical evaluation and self-report with regards to safety being accurate. In the proposed study, staff will check question 9 of the BDI-II regarding self-harm before the participant

leaves the evaluation. Participants will also have a C-SSR at screening and week 4, 8, and 12. The screening, week 4, 8, and 12 evaluations are done by research staff defined as being trained and certified to assess for safety to self and others (e.g., MD, PhD, LICSW, Master's level therapist). In addition to the monthly evaluations, subjects also fill out weekly adverse event sheets that ask if they symptoms of depression are worse or if they want to harm themselves. If increasing symptoms of depression are endorsed, this will trigger an evaluation by a research staff or the PI. Some fluctuations in depressive symptoms are expected and are not reported as adverse events unless there is a change in the level of severity as recorded in the adverse event clinical reporting forms.

4.6.1.11: MRI Risk Protection: We have a detailed MR Safety Screening Form that every person must complete before he/she receives a MR scan. All participants with ferrous metal near vital structures will be excluded from participating. At the time of the scan, participants will be asked to remove all metal objects from their person, including watches, jewelry, change, wallets, credit cards, and shoes, which will be kept by the research team in a safe location. Participants are also checked with a metal detector prior to entering the scanner. The FDA has determined that MRI machines of 4.0 or less do not represent significant radiation risk. Radio Frequency (RF) power limits will conform to the FDA guidelines defined in IEF 60601-2-33, the international standard for the safety of magnetic resonance devices intended for medical diagnosis. Participants are offered earplugs and a set of earphones to reduce the noise level. Blankets and pillows are used to make the subject more comfortable. There is a two-way audio system in the scanner through which the research team and the participant can communicate at all times. Although the experience of being in the scanner can be uncomfortable, it is our experience that a majority of subjects tolerate the procedure quite well. Subjects with clinically significant claustrophobia will be excluded from this study.

4.6.1.11.

4.6.1.12. **Psychological Risks Protection:** A member of the research team will be available to answer questions concerning procedures and psychological tests at all times during the study sessions. The practice of yoga or walking interventions is unlikely to cause adverse psychological reactions. The exception is that subjects with a history of trauma may find the yoga postures elicit memories of the trauma. All subjects are screened for trauma and those with a history of PTSD or PTSD symptoms are monitored for increasing symptoms during the study. The yoga instructors will be instructed on methods for addressing the special needs of trauma victims. Subject with dissociation due to PTSD could be excluded at the discretion of the Principal Investigator. In the unlikely event that a subject has an adverse experience during an intervention session, clinical staff in addition to the yoga instructors will be available during the interventions to assess subjects who are in distress. The inclusion criteria are designed to identify subjects with MDD who are not a danger to themselves or others and who are a low risk to have an adverse reaction to the interventions. As participants are Veterans and entitle to care form the VA, if there is a risk of harm to self that would require hospitalization, they will be taken to the walk in clinic or the ER for further evaluation.

4.6.1.13. **Alcohol Risk Protection:** In order to prevent subjects under the influence of alcohol from participating in evaluations or the interventions, all subjects will have a Breath Alcohol Concentration (BAC) done after the written informed consent and before participation in the weeks 4, 8, and 12 evaluations and at any evaluation/intervention if research staff are concerned a participant is under the influence of alcohol. If the BAC reading is not zero, participants will not be able to participate in the evaluation/intervention. If the BAC is above 0.08% or 0.08 mcrg/l, the level of legal intoxication in Massachusetts, the participant's car keys will be taken until their BAC is less that 0.08 %. The BAC will be done with a hand held Alcosensor machine Subjects will be assessed by clinical staff for safety prior to leaving if their BAC was greater than zero. These procedures are outlined in the informed consent such that participants are aware of these procedures prior to signing the informed consent.

4.6.1.14. **Physical Risks Protection:** To facilitate the evaluation of a subjects ability to safety comply with the interventions, they will as part of the inform consent process allow study staff to review their

VA medical records as needed. If there is a question concerning a medical problem that would be a contraindication to participation that is not in their VA medical record, the Veteran will be asked to sign a release of information to allow contact with their medical care clinicians to resolve the issue. If the Veteran declines, they maybe excluded from the study. All subjects will be assessed by a staff physician for a history of prior injuries or medical conditions that would preclude the subjects from safe participation in the yoga intervention. All subjects will have a physical examination at the screening visit as part of their safety assessment. The yoga intervention for the proposed study was piloted in Study 2 and 4 and was associated with minimal adverse events. This study uses certified yoga Instructors with Iyengar training and at least 5 years of teaching experience. Iyengar yoga is designed so that adaptations are made on an individual basis using position modifications and props such as blocks, blankets and belts. This individualized approach allows physical limitations of the subjects to be addressed and reduces and/or minimizes the risk of injury and muscle soreness. The Faros Bittium will be used in accordance with the manual. Staff that has been trained to perform the procedure will do the placement and removal of the electrodes. The Principal Investigator who is boarded in neurology and used to work at a Rehabilitation Hospital will screen subjects for difficulty with the Step Test prior to randomization.

4.6.1.15. **Protection of Special Populations:** Individuals with psychiatric illness are considered special populations. This study will enroll subjects with MDD without psychomotor depression, psychosis, or agitation that would prevent participation in the study. This level of depression should not affect cognition to the degree that they would be unable to consent to the study. Psychiatric disorders that would impair the ability of subjects to consent such as bipolar disorder, psychosis and dementia are exclusionary. All subjects take a quiz after signing the consent to assess if they understand the risks and benefits of the study. A subject may be excluded if he/she make errors on the quiz and cannot demonstrate that he/she understands the error. Yoga is safe for use in pregnant females; in fact yoga is being studied as a potential treatment for perinatal depression. Accordingly there is minimal risk to a pregnant participant or the fetus from participation in this study. However the presence of pregnancy would be a confounding variable as the risk of depression can increase during pregnancy. For the integrity of the study, pregnant females are excluded. In order to avoid unnecessary procedures, females will be asked if they are pregnant or intend to become pregnant and are required to use an acceptable form of birth control during the study. Only if there is a change in their normal menstrual cycle such that pregnancy is a concern will females be considered for exclusion. All females of child-bearing capacity must use one of the following approved methods of birth control: oral contraceptives ("the pill"), contraceptive implants under the skin, contraceptive rings or patches or injections, diaphragms with spermicide and condoms with foam, surgical sterilization, or complete abstinence from sexual intercourse. IUDs with progesterone implants are not allowed because they can cause anovulation. IUDs with metal are not allowed due to the MRI scans.

4.6.1.16. **Confidentiality Risk Protection:** Information is kept in separate databases. 1) To ensure confidentiality, all information on clinical report forms (CRFs) or placed directly into REDCap will be marked with subject IDs so that it cannot be associated with any individual. 2) A master list, with individual names and their subject IDs, will be kept in a locked file cabinet or password-protected computer file that can be accessed only by research staff. 3) Identifiable information is kept in a third database. The information gathered will only be used for scientific, educational, or instructional purposes. Exceptions to this rule will be made for representatives of the FDA, other U.S. government agencies, and other mandatory bodies as required by law. Because of the sensitive nature of the questions surrounding the use of illegal substances, a Certificate of Confidentiality will be obtained. While subjects are instructed that the groups are confidential, by the nature of conducting the classes in a group the confidentiality of what is said in the group cannot be guaranteed. For this reason, subjects are instructed to monitor what they say in the group settings. Subjects are informed that they can ask to speak to a staff member in private.

4.6.1.17. **COVID-19 Risk Protection**: In addition to the already approved safety measures for when

the participants are at the VABHCS additional measures to increase social distancing have been added. The change of the in-person interventions to virtual interventions except for the randomization, and weeks 4, 8, and 12 evaluation is a change that could affect the scientific integrity of the study. As no subject had started the interventions prior to the shutdown of all non-essential research in March 2020, all subjects will have the same experience of virtual interventions. The Veterans Administration Central Office (VACO) Project Manager has given tacit approval to this change, provided it is IRB approved and the documents reflecting VACO approval of the changes are filed by the VABHCS ACOS with VACO. The changes to the intervention to make them virtual have been listed in the protocol. The video connections will be made with VA Video Connect or another platform approved by the VABHCS Information System Security Officer (ISSO).

4.6.2. Data Safety Monitoring

4.6.2.1. **Data Monitoring Committee (DMC):** Clinical Science Research and Development (CSR&D) has a centralized Data Monitoring Committee (DMC) that monitors clinical studies that are approved for funding, include human subjects. The CSR&D DMC provides an ongoing independent evaluation of the progress of studies, including participant accrual and retention, adverse events monitoring, and analysis plan. Loss or theft of data is considered an adverse event that would be reported to the DMC. The DMC is a service that is provided by CSR&D to ensure independent oversight of the safety and integrity of the project. No other DMC or Data and Safety Monitoring Board (DSMB) review is needed. The DMC office is located within the Cooperative Studies Program Coordinating Center (CSPCC) at the Edward Hines, Jr. VA Hospital in Hines, IL.

4.6.2.2. **Suspension of the Study and Breaking the Blind:** The DMC that has the authority to suggest suspension of the study if subject safety is at risk. The final decision regarding suspension lies with VACO. Because the subjects are not blinded to the yoga or walking intervention they are receiving, a protocol to break the blind concerning group assignment is not required. As was done in March 2020, the study maybe suspended due to an order from VACO related to the COVID-19 pandemic.

4.6.2.3. **Data Storage and Period of Data Retention:** Hard copies of research data will be kept according to VA regulations. Computer Files will be locked and maintained according to hospital and research standards. All computer data will be stored on a firewall-protected server. Computer data will be password-protected and access will be restricted to research staff and the Principal Investigator. The informed consents have a section giving permission for the data to be used "to study the effects of yoga on disease, health and well-being" such that analysis of the data that falls under this description has already been agreed to by subjects. The approved IC already documents that if a data repository is created in the future subject consent has been obtained. If subjects do not check this box, their data will not be used for to additional analysis. Currently, VA regulations require that data be retained for 5 years after the end of the fiscal year in which the project is closed at which time the data will either be destroyed or put into a repository. Permission to create a data repository that combines the data from the Merit Award, the R01 and R21 on which the Merit Award is based is requested.

4.6.2.4. **Compliance with VA Regulations:** The REDCap database and the final dataset will be a Limited Data Set that contains only the date of data collection. HIPPA identifiers include the data of data collection, but these dates it will not allow reasonable identification of the subjects.

The Azure Rights Management Services (RMS), system that has been approved for use by VA researchers to send information to both subjects and other researchers. After the original contact, potential participants will be given an option to communicate via VA approved secure email system designed for patients/participants use. Besides contact information and dates of service, no personal health information will be released by email. Subjects will be instructed in how to use Azure to send and receive data. Azure

RMS will also be used to transfer a blinded dataset of electrocardiogram data to Dr. Heilman in the University of North Carolina for analysis. The only Private Health Information (PHI) that will be transferred is this limited data set are the dates of the intervention. A user guide will be provided to Dr. Heilman detailing the proper procedures for replying to and sending data as an Azure RMS protected message.

4.6.2.5. **Protection of Data:** Study data will be collected using the of REDCap data collection system, a software tool developed at Vanderbilt University and made available through the Clinical and Translational Science Awards network (CTSAs). To help protect and secure the data stored in REDCap's database, the software application employs several methods to protect against malicious users who may attempt to identify and exploit any security vulnerabilities in the system. Access to the REDCap data entry website will be based on permissions granted by username and password. Only authorized study members will be able to enter or view data. The login information (username) of the person submitting the information, the date and time submitted, and other navigational information will be automatically obtained and stored in the database. When available, staff will sign subjects into a secure link to access REDCap, such that subjects will have access only to required assessment forms that they will fill out in REDCap:) Beck Depression Inventory (BDI-II), Spielberg State-Trait Anxiety Inventory (STAI), PTSD Check List – Civilian (PCL-C), Exercise-Induced Feeling Inventory (EIFI) and Pittsburgh Sleep Quality Index (PSQI).

4.6.2.6: Data on Hard Drives: It is anticipated that the study will be provided with three computers.. These computers will be encrypted. It is anticipated that the study staff will be provided with mobile devices, one for the PI, one for the project manager and and one for the clinical staff, these devices will comply with encryption technology that is FIPS 140-2 validated.

4.6.3. Potential Benefits

4.6.3.1. **General Benefits:** All subjects will have the benefit of an evaluation of their depressive symptoms by clinical staff. Subjects, who have a MRI scan at the McLean Hospital, will have the advantage of having the anatomic study read as part of the safety protocol.

4.6.3.2. **Benefits of the Yoga Intervention:** The yoga intervention in this protocol includes yoga postures and yoga breathing. Both practices have been shown to have health benefits in controlled trials of normal subjects and clinical populations. In addition to the benefit of physical activity, the yoga intervention includes exercises for flexibility, strength and balance. Yoga practices have been associated with stress reduction, weight reduction, improved sleep, and decreased blood pressure to name a few of it's health benefits. Yoga practices have been shown to decrease symptoms in disorders with low GABA activity such as depression, post traumatic stress disorder (PTSD), epilepsy and chronic pain. There have been controlled studies showing yoga-based interventions to be effective in treating depression ranging from mild depression symptoms to major depressive disorder. The benefits to the subjects participating in this study include the both the general health benefits of yoga and the potential to treat the depressive symptoms.

4.6.3.3. **Benefits of the Walking Intervention:** There is an extensive literature that exercise improves mood such that the walking intervention is an active control. The walking intervention includes the benefit of regular exercise.

4.6.3.4. **Additional Benefits:** Social interaction with the research staff and the group members in a population that can be socially isolated is a benefit. Additional benefits include an evaluation by a physician who will discuss treatment options if the subject does not participate in the study. Subjects are closely monitored during the study for increasing symptoms of depression.

IMPORTANCE OF KNOWLEDGE TO BE GAINED

4.6.3.5. **Understanding the Relationship Between Yoga Practices, Parasympathetic Tone and Mood:** Integrated medical practices such as yoga and exercise are becoming increasingly popular. It is critical that the mechanisms through which a yoga practice confers benefits in the form of reduced stress and improved affective states be elucidated. This study will examine the relationship between yoga, the activity of the parasympathetic nervous system and mood. Decreased parasympathetic tone has been documented in a number of psychiatric diseases, with low high frequency heart rate variability (HRV), a marker of low parasympathetic tone being a marker for overall mortality. If this study confirms the findings in the Preliminary Data Section that a yoga intervention is associated with increased parasympathetic tone and improved mood in a Veteran population, it would have significant implications for treatment of disorders with low parasympathetic tone such as depression, PTSD, and Alcohol Use Disorder, all disorders with a high healthcare burden in the Veteran population. **Reduction in Suicidal Ideation:** There is currently significant concern about the rate of suicide in the Veteran population is higher than the civilian population. The proposed intervention resulted in the elimination of suicidal ideation without intent present at screening in a prior dosing study in 9 out of 10 subjects, or by approximately 30% in the population that completed the study (9/30).

4.6.3.6. **Use of Yoga Intervention as Monotherapy or as an Adjunct for Partial Remission:** The development of an intervention that can be used as monotherapy and as an adjunct to other treatments for depression that has the documented ability to decrease suicidal ideation would be an advance for a field in which up to 40% of those treated do not achieve remission. The Preliminary Data Section shows improvement of depressive symptoms in subjects on a stable dose of monoamine therapy when the proposed yoga intervention was added to their treatment. A better understanding of the neurophysiology underlying the practice of yoga could advance the treatment of many disease processes, as well as reduce the costs to the afflicted individuals, the health care system, and society

4.6.4. Alternative Procedures

The alternative is to not participate in the study. Participants may choose to seek standard care for their depression such as psychotherapy, medications electroconvulsive therapy (ECT), and transmagnetic stimulation (TMS).

4.7 Costs and Payments

Subjects will be paid for their time: $10 for each group session, $20 for the week 4 evaluations, $40 for the week 8 evaluations, $60 for the week 12 evaluations, and $40 for each MRS scan for a maximum of $480. Payments are made one or twice during the study, depending on the participants request via check. Subjects are not required to pay for services outside of the VA with the exception as to whether they choose to drive to the McLean Hospital, were parking is free or decide to take public transportation to the McLean Hospital. If a subject provides proof of the cost for transportation (e.g., taxi, Uber, or Lyft) on the day he/she has a scanning session, the participant will be compensated for his/her the cost of travel at a rate of $40 for each leg of the journey, or $80 round trip. If participants decide to keep the yoga blocks after the intervention, $20 will be deducted from their payment. Subjects are paid for the scans at the McLean Hospital as long as they are compliant with the protocol (e.g., As they are instructed not to drink prior to the scan in the informed consent, if they arrived for the scan under the influence of alcohol they would not be paid. If there were a scan malfunction, such that the subject arrived and could not be scanned, they would be paid for one scan. If they agreed to come back they would also be paid for that scan).

4.8 Providing for Reuse of Data:

The data will be also be stored in the Boston Yoga Research Center (BYReC) data repository being set

up here at the VABHCS, for future research studies pertaining for future research studies pertaining to the effects of yoga and walking on disease, health, and well being. All data will be stored and maintained according to VA regulations and only investigators approved through the Social and Community Reintegration data repository committee will have access to this data. Any future use of the data will be reviewed and approved by this committee. The creation and management of this data repository will be approved by the hospital IRB and Research and Development Committee before any of the data from this study will be stored in it for future use. Once the Data Repository has been approved, an amendment to this project will be submitted to store the data in the data repository.

4.9 Creation of a Tissue Bank: No Tissue Bank will be created

5.0 RESOURCES

This is a research study such that all evaluations, interventions, intervention assessment and laboratory testing for serum progesterone and pregnancy test will be done as part of this research study. The imaging component of this study will be done via a contract at the McLean Hospital. This study is staffed for the PI to have a 3/5th time position with the VA. There is a full time project manager and a 50% time clinical staff at the ENRVA Hospital. Dr. Chun Zuo, a Co-Investigator with an appointment at the McLean Hospital, will oversee imaging acquisition and the analysis of the spectral data. Dr. Keri Heilman is an expert in analysis of ECG readings for analysis of RSA. Dr. Richard Brown and Dr. Patricia Gerbarg are experts in the use of alternative treatments for psychiatric disease and will serve as consultants is there are clinical concerns regarding the patients.

Dr. Streeter has the following space in Bldg. 70, two offices, 119 and B31, the use of a large room for the in-person yoga intervention and a large hall with low traffic for the in-person walking interventionThe clinical staff will use one of the offices on the first floor for the week 4, 8, and 12 assessments, while the interventions will take place on the basement level, this separation will decrease the likelihood that clinical staff will be exposed to subjects during the intervention such that they would no longer be blinded to the interventions.

A. BEDFORD VETERANS ADMINSTRATION MEDICAL CENTER

Bedford Veterans Administration Medical Center (VAMC) serves a large number of Veterans with mental illness who are and are not in treatment and thus provides a clinical population that would both benefit from the proposed study and will allow recruitment of the require research subjects. The resources below outline the extensive services and resources available at the Bedford VAMC.

I. CLINICAL RESOURCES

Inpatient Psychiatry**:** Bedford has an acute inpatient psychiatry unit (30 beds) as well as a sub- acute unit (22 beds). Both programs have incorporated a recovery philosophy that orients Veterans to the goal of community integration as eventual outcome for all treatment. Peer specialists and community-based activities have been incorporated into the programs over the last several years.

Outpatient Mental Health Clinic: The outpatient clinic serves 5,000 Veterans per year, providing the full range of outpatient mental health services. In 2015, there were 3,971 patients receiving treatment with antidepressant medications. The clinic staff is organized into five interdisciplinary teams consistent with the Behavioral Health Interdisciplinary Program (BHIP) model, and has served as an exemplar of the BHIP program. The Mental Health Clinic provides services through three Community Based Outpatient Clinic (CBOC)'s in Gloucester, Haverhill, and Lynn, Massachusetts.

Specialty Treatment Programming for Substance Use Disorders: The Veterans Mental Health and

Addictions Program (VMHAP) is an intensive day treatment program for substance use disorders. It serves 500 Veterans per year. The Aftercare Program provides a range of outpatient services for substance use disorders to 300 Veterans per year.

Integrated Mental Health and Primary Care Program: The Integrated Mental Health/Primary Care Program uses a co-located model of care to support the provision of mental health services by primary care providers and to facilitate the transition of some Veterans to specialty mental health care.

II. RESEARCH

Previous Research: The Outpatient Mental Health Clinic has been the site of numerous prior research studies with excellent staff support and interest. Both in-patient units have been the sites for prior research studies. There is a history of meditation and yoga-based research studies being conducted at the Bedford VAMC with high interest from the patient population.

The Social and Community Reintegration Research (SoCRR) is a VA Rehabilitation Research and Development (RR&D) Reserve Educational Assistance Program (REAP) located at Bedford, bringing together a diverse group of VA researchers as a consortium to address the urgent need to build capacity for high quality research in the priority area of community reintegration for Veterans with psychiatric disabilities. Veterans with psychiatric disabilities are often withdrawn from or are at risk for having difficulty sustaining community involvement because of the effects of their psychiatric disabilities. SoCCR's focus on critical areas of community reintegration, including employment, education, and interpersonal relationships, converge on the central mission of helping Veterans become more engaged in needed treatment that targets reasons for poor community involvement (e.g., difficulty maintaining jobs or education, relationship difficulties, low social support).

The New England Mental Illness Research Education and Clinical Center (MIRECC) is co-located at the Bedford and West Haven VA's. The New England MIRECC focuses on rehabilitation research and educational services designed to address the needs of Veterans with combined mental illness and drug or alcohol dependence (dual diagnosis). The aim of the MIRECC is to improve the treatment of dually-diagnosed Veterans by developing innovative new treatment for co-morbid substance use disorders, devising more effective ways to deliver existing treatments, and creating better programs to train VA treatment providers in therapies with proven efficacy. The research program is comprised of an interrelated series of studies at three levels: a) health services research, b) applied clinical research and c) basic clinical research on the neurobiological basis of dual diagnosis.

The Center for Healthcare Organization and Implementation Research (CHOIR) is co-located at the Bedford and Boston VA Medical Centers, and is one of the 19 Centers of Excellence within the Veterans Administration Health Services Research and Development Program. The Center's mission is to improve Veterans' health outcomes by developing, studying and applying evidence-based practices that will be widely implemented and sustained. The three research priority areas are: Recovery in behaviorally vulnerable populations, Medication optimization, and Public health communication.

The National Center on Homelessness Among Veterans: With researchers located at the Bedford and VA Connecticut Healthcare System campuses, this Center seeks to promote recovery-oriented care for Veterans who are homeless or at risk for homelessness. The Center is a national resource that conducts research to inform practices tied to the VA five-year plan to end homelessness among Veterans. The Center has four resource cores: Policy Analysis, Model Development & Implementation, Education & Dissemination, and Research & Methodology. The Boston University Center for Psychiatric Rehabilitation**,** located in Boston, Massachusetts, has been primarily a training collaborator with staff at Bedford, and has assisted VACO in guiding VA psychiatric rehabilitation services development. The Center is affiliated with the Sargent College of Health and Rehabilitation Sciences at

Boston University. The Center is a research, training, and service organization dedicated to improving the lives of persons who have psychiatric disabilities by improving the effectiveness of people, programs, and service systems. Its work is guided by the most basic of rehabilitation values that first and foremost, persons with psychiatric disabilities have the same goals and dreams as any other person. They want a decent place to live, suitable work, social activities, and friends to who to they can turn in times of crisis. The mission of the Center is to increase knowledge in the field of psychiatric rehabilitation and to apply this body of knowledge to train treatment personnel, to develop effective rehabilitation programs, and to assist in organizing both personnel and programs into efficient and coordinated service delivery systems.

The Boston University School of Medicine (BUSM), Department of Psychiatry, located in Boston Massachusetts is the primary academic affiliation for the Bedford VAMC. Many of the Bedford VAMC psychiatry staff has academic appointments at the BUSM. The department's residents and addiction fellows rotate through the Bedford VAMC inpatient units and outpatient clinics. This affiliation provides additional support for research endeavors at the Bedford VAMC.

III. PROGRAMS FOR VETERANS RECEIVING MENTAL HEALTHCARE

The following programs are potential sources for recruitment.

The Community Reintegration Program is a national exemplar, designed specifically to help Veterans currently engaged in mental health care at the Bedford VAMC to become more fully engaged in community activities. The program is staffed by four full-time peer specialists and one psychologist. Each peer is assigned to a geographical area where many of the Veterans served at Bedford either live or want to live once treatment is complete. Each peer has developed relationships with community stakeholders and resources in key categories: (1) special Veteran groups (peer support groups, Veterans of Foreign Wars (VFW), Disabled American Veterans (DAV), etc.); (2) other peer support groups (non-Veteran peer support groups, Alcoholics Anonymous (AA), Narcotic Anonymous (NA), etc.); (3) local Veteran-specific programming (town Veteran Services Officers, Veteran housing and employment specialists, etc.); (4) recreation groups (local softball leagues, etc.); (5) religious and spiritual communities, including those with significant numbers of Veteran members; and (6) individual Veterans in the community who are willing to support and mentor Veterans transitioning out of care.

Bedford Vocational Services: The Bedford Vocational Services program (also called Compensated Work Therapy or CWT) has been one of the largest VA vocational programs in the country for many years, and has been a national leader in innovative programming. Supported Employment (SE), Transitional Work Experience (TWE), and Incentive Therapy (IT) are all vocational rehabilitation programs supported at the Bedford Campus. Over 300 Veterans are admitted to the program each year. Approximately 150 Veterans receive Supported Employment per year, making Bedford one of the largest VA providers of Institute of Psychological Services (IPS) Supported Employment. Approximately 130 Veterans participate in Transitional Work Experience, the VA's traditional model of vocational services. These Veterans work in the medical center and in the community while they prepare for returning to competitive employment. Supported Self Employment is an adaptation of IPS Supported Employment, designed specifically for Veterans interested in self-employment. The program serves 50 Veterans per year and has been now successfully disseminated to the West Haven VA, Boston VA, Manchester and Brockton VA's. The Supported Education program is one of less than 10 in the VA nationwide, and represents a new intervention designed to serve the education needs of returning Operation Enduring Freedom/Operation Iraqi Freedom (OEF/OIF) Veterans.

Peer Services**:** Certified peer specialists represent a key resource for encouraging community integration/reintegration, as they model the successful return of Veterans with mental health problems to full community involvement. Bedford's program has been identified by VACO as a national exemplar, with 13 full-time Certified Peer Specialists working in virtually every mental health program and in Primary Care. Peer specialists provide a range of services, including introducing Veterans to programs, helping them navigate the VA system, providing informal support, modeling successful functioning, and running peer support groups. Strategically, they are among the most important staff during the transition from active engagement in treatment to full community re-integration. The Bedford Peer Services Program is highly involved in the VACO peer services program and offers education and training to peer specialists from around the country through the Mental Illness Research Education Clinical, Centers of Excellence (MIRECC) Peer Education Center.

The Safing Center is a specialty outpatient mental health program that provides consultation, crisis management, and individual and couple therapy to Veterans who are using and/or experiencing violence in their intimate relationships. Additionally, the Safing Center provides outreach, consultation, and education and training, services to staff and Veterans, locally, regionally, and nationally. The Safing Center approaches relationship violence from a comprehensive, Veteran-centered, recovery-oriented framework, which includes attention to all domains of health (physical and mental health, relational health, occupational/educational health). The Director of the Safing Center has pioneered new interventions for (1) identifying and (2) treating Veterans who are using and/or experiencing violence in their intimate relationships. The Safing Center has been designated as a national exemplar by VACO, and interventions developed at the Safing Center are being disseminated by VACO to 5 other pilot sites.

Other VA Mental Health Rehabilitation and Recovery Programs**:** Bedford has been a national leader in developing recovery-oriented rehabilitation programs for Veterans with mental illness. Bedford has some of the largest rehabilitation services in the nation including one of the largest vocational programs, the largest Transitional Residence (serves 80 Veterans per year), one of the largest Mental Health Intensive Case Management (MHICM)'s (serves 120 Veterans per year), and one of the largest Veterans Administration Supportive Housing (VASH) programs (serves 200 Veterans per year). Virtually all of these programs have been sites for prior research studies and staff has been good partners in VA research efforts. The MHICM program has been a national leader in Acceptance and Commitment Therapy (ACT) programming supported by relationships with mental health professionals and has been designated a national center of excellence. The Veterans Community Care Center is a Psychosocial Residential Rehabilitation Treatment Program (PRRTP) program designed to help Veterans with Serious Mental Illness (SMI) who are living in the community, expand their independent functioning in that community. The Domiciliary Care Program is a 50-bed Residential Rehabilitation Treatment Program (RRTP) program built around the goal of helping homeless Veterans struggling with mental illness and addiction, to rebuild their lives in a community of their choice. The program works closely with Vocational Services and the Community Reintegration Program to help Veterans re-establish full independence in the community. The VASH program was a pioneer in developing the VASH model of homeless intervention, and has been a site for Critical Time Intervention studies. The CWT/TR has been the site of studies of efficacy of TR programming and of enhancements like contingency management.

Office Space:

Dr. Streeter has the following space in Bldg. 70 to conduct the study, two offices, 119 and B31, the use of a large room for the yoga intervention and a large hall with low traffic for the walking intervention. There are additional conference rooms that could be scheduled for patient interviews as needed.

Gymnasium and Pool:

The Bedford VAMC has a large gym, pool and exercise areas that can be used for the interventions.

Computer:

Computer services and equipment will be provided by the VA Information technology office.

6.0 Collaborations

There will be a contract with the McLean Hospital for the imaging component of this study. The goods and services will include scan acquisition, scan analysis and the associated costs for McLean Staff that will include Dr. Zuo, Rose Villefuerte, MRI scan technicians and a to be announced research assistant.

7.0 Qualifications of the Investigators

Personal Statement for Key Personnel and Consultants

Chris Streeter, M.D.: Principal Investigator

I have worked in the VA system for over 15 years and have extensive experience in treating Veterans with psychiatric and neurologic illness. I will have a 3/5th time appointment at the VABHCS when this study commences. Either I or the other clinical member of the team to be announced will be responsible for the study related healthcare decisions.

For the past 40 years I have pursued studies that explore the mind-body interface. Starting in college with Southeast Asia Studies, a neurology residency, a behavioral neurology fellowship and a psychiatry residency and as a long time yoga practitioner. I have acquired the skills required to be the Principal Investigator for the proposed study. As the Director of Functional Neuroimaging for Psychiatry and the Director of the Boston Yoga Research Center, I used magnetic resonance spectroscopy (MRS) to measure gamma aminobutyric acid (GABA) in cocaine dependent individuals. I observed that depression, anxiety and epilepsy had symptoms that increased with stress and decreased when treated with pharmacological agents that increased brain GABA levels, and that yoga interventions also improved symptoms in the same disorders. This lead to the hypothesis that yoga in part acted through the GABA system, which was refined by the including yoga's effect on the parasympathetic nervous system. This hypothesis was supported by 4 prior studies confirming the association between a yoga session, increased parasympathetic tone, increased brain GABA levels and improved mood. I have an appointment at the McLean Hospital and have been the PI on previous studies were MRS was used to measure brain GABA levels. This Merit proposal extends this research program to include a Phase 2 Randomized Controlled Trial of the manualized yoga and breathing intervention and walking control developed in prior R21 and R01 studies in civilians to Veterans with Major Depressive Disorder I have experience in leading multidisciplinary teams and have been a Co-Investigator in nine clinical trials sponsored by the National Institute of Health (NIH) and industry. I and my team are devoted to the development of rigorous evidence based studies to guide the use of the proposed yoga and breathing intervention for the treatment of Veterans with Major Depressive Disorder in the short term with the expansion to other disorders and efficiency studies in the long term. The publications listed below document previous research in Veterans regarding Post Traumatic Stress Disorder, Alcohol Dependence, Traumatic Brain Injury, and all topics important to Veterans and relevant to the proposed protocol.

Keri Heilman, Ph.D.

My primary research interests focus on investigating how physiological state influences social behavior. I am specifically interested in identifying the neural mechanisms mediating inappropriate social behaviors. The long-term goal of my research is to develop tools that could use biobehavioral indices as early predicators of psychopathology. I have been a consultant on Dr. Streeter's Study using

yoga to treat depression, where I assisted in the study design with regards to the collection and analysis of the Respiratory Sinus Arrhythmia data. I am well qualified to serve as a consultant on the continuation of this research in Veterans.

During the course of my research career working with Dr. Stephen W. Porges, I have become proficient in the monitoring of physiological indices of social behavior and emotional regulation, such as heart rate variability, eye gaze, facial muscle activity, auditory processing and cortisol. I have been developing my specific area of expertise – the collection, editing and analyses of heart rate data for cardiac rhythms - since 2000. I have been regularly teaching training workshops on the editing and analyses of heart rate data since 2008.

I have experience working with participants of all ages (infants through adult). I have conducted research to investigate how difficulties in behavioral state regulation in infants and toddlers (e.g., fussiness, crying, tantrums) are related to developmental trajectories. I have additionally worked with a variety of clinical populations who share a difficulty in social behavior/emotion regulation, such as children with autism spectrum disorder, social anxiety disorder and selective mutism. More recently, I have been involved in research studies that evaluate the effects of trauma in children and adolescents, and the effects of a biobehavioral intervention on autonomic/behavior regulation and auditory processing.

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