Acupuncture for Combat PTSD: A Clinical Trial

Protocol

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I. Background

The Significance of PTSD and its Psychiatric Comorbidities in the Study Population

Posttraumatic Stress Disorder (PTSD) is a debilitating disorder characterized by re-experiencing aspects of the original trauma, avoidance and numbing of trauma reminders, negative alterations in cognition and mood, and hyperarousal (APA, 2013). Lifetime prevalence of PTSD in community samples is about 6.8% (Kessler et al, 2005) and as high as 30% in Vietnam Veterans (Kulka et al, 1988) and rape survivors (Resnick et al, 1993). Recent OEF/OIF/OND Veterans have 13-16% PTSD prevalence, dependent on combat exposure (Litz et al, 2009). Personnel were at high risk for severe injury and for witnessing or suffering from the aftermath of violence (Ricks, 2004). Of soldiers in Iraq, 86% reported knowing someone who was seriously injured or killed, 68% saw dead or seriously injured Americans, and 51% handled human remains (Hoge et al, 2004). Active and reserve soldiers report similar rates of combat experiences (67% vs. 70%) (Milliken et al, 2007).

PTSD is highly comorbid (83-90%) with other psychiatric disorders, including mood, substance use, personality disorders, depression, and panic disorder (Bradley et al, 2005). Approximately 50 to 60% of PTSD patients have major depressive disorder (Bradley et al, 2005). This clinical comorbidity parallels shared biology. Neurological mechanisms of PTSD, depression, and anxiety disorders have many similarities (Rechlin et al, 1995). The known genetic variance for depression and anxiety disorders accounts for the majority of known genetic variance for PTSD (Koenen, 2007). Because of the high clinical and biological comorbidity, choosing the most appropriate inclusion and exclusion criteria for the proposed study is challenging. Excluding potential subjects with comorbidity markedly reduces feasibility and generalizability. For these reasons, most clinical studies limit exclusion of comorbidities (e.g., (Schnurr et al, 2003). Selection criteria are meant to recruit a sample with chronic PTSD and common comorbidities, yet without severe depression or other comorbid conditions that would likely confound treatment and biological assessment.

Interventions for PTSD

Evidence Based Pharmacotherapy and Psychotherapy. A recent review noted strengths and limitations of interventions that have evidence of efficacy for PTSD; references for this are in the publication in Appendix 3 (Hollifield, 2011). Ipser and colleagues published a recent review and meta-analysis of medication RCT's (Ipser et al, 2012). The meta-analysis included 23 trials that met investigators inclusion criteria (direct placebo comparison). Overall, active medications resulted in a significant, clinically modest reduction of symptom severity on the Clinician Administered PTSD Scale (CAPS) (Blake et al, 1995) (-6.10 points, n=4112). Paroxetine provided the largest reduction of CAPS scores (-10.65 points, n=1100), sertraline showed modest effects (-4.35 point, n=1260), and paroxetine was significantly more effective than sertraline (fixed-effects model: $X2=10.37$, $p=0.001$). Psychotherapy interventions include a group of therapies under the rubric of cognitive behavior therapy (CBT), somatic experiencing, and mindful/spiritual approaches. Trauma-focused CBT such as prolonged exposure and cognitive reprocessing provides significant treatment effects. However, trauma-focused CBT has limitations, such as high rates of non-engagement in treatment, high withdrawal rates, and a significant risk of becoming more symptomatic. Interventions that minimize exposure to trauma content such as imagery rehearsal therapy and stress inoculation training also have evidence of efficacy for PTSD.

The Demand for Other Approaches Including Acupuncture. A national survey in 1997 showed that 42% of individuals used one of 16 complementary/alternative medicine (CAM) therapies in 1 year (Eisenberg et al, 1998). Most commonly used were herbs/supplements, massage, chiropractic, spiritual healing, acupuncture (ACU). It was estimated that 4% had used ACU. Similarly, a large survey conducted by the Samueli Institute with the Department of Defense showed that $\geq$45% of active-duty military members used any CAM per year. ACU is commonly used by active-duty military and Veterans for PTSD. The HAIG 2010 survey reported that 25% of VHA facilities offered acupuncture service onsite and 16% referred to non-VA providers, which implies that about two-fifth of VHA facilities offered acupuncture service or referred veterans to non-VA providers.

Acupuncture: Our Developmental Work. Given the potential value of interventions that minimize trauma exposure coupled with research showing that acupuncture had effects on symptoms related to PTSD, we developed an acupuncture intervention for PTSD. For a review about the efficacy of acupuncture for anxiety, depression, and insomnia see publications in Appendix 3 (Hollifield, 2011; Hollifield et al, 2007). The first step was to develop a standardized intervention for PTSD based on Traditional Chinese Medicine (TCM) practice. We conducted a textbook review of the potential points to use for symptoms of PTSD, an expert survey to obtain opinions about points to use, and then pilot diagnosis and treatment planning with 22 PTSD patients. The primary TCM disease patterns that best described PTSD were Heart Shen disturbances, Liver Qi (energy movement) stagnation, and deficiency in the Kidney system. An explanation of these disturbances and the treatment points developed for our trial is in our published report (Sinclair-Lian et al, 2006).

PTSD Involves Complex Biology: Choosing Biological Outcomes

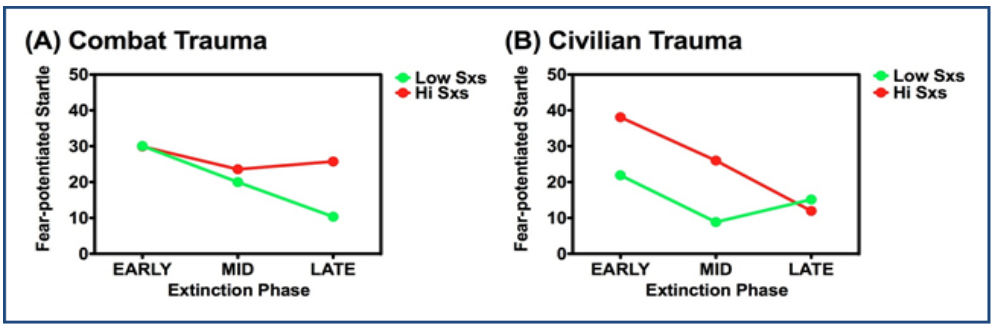
Major summative findings indicate complex biology of PTSD including HPA axis dysfunction, autonomic nervous system (ANS) dysfunction, alterations in central nervous system (CNS) processes, and inflammatory dysregulation, all under complex gene – environmental interaction control (in (Hollifield, 2011), Appendix 3).

Focus on ANS Peripheral Psychophysiological Responses (PPR). Detailed below, there is consistent evidence that indices of PPR we propose to use in this study distinguish PTSD from trauma control subjects, may change with acupuncture, and are good translational tools to assess fear conditioning and extinction.

Theoretical Framework: Failure of Extinction is Central to PTSD Pathology. PTSD symptoms are thought to be classically conditioned by exposure to aversive sensory stimuli (unconditioned stimuli: US) during combat trauma that provoke a fear/stress response (unconditioned response: UR). Similar sensory stimuli in subsequent non-combat situations, such as fireworks (conditioned stimuli: CS) provoke the fear/stress response (conditioned response: CR). Over time, the CR/PTSD symptoms generalize because the CS and CR occur in a high number of daily situations. These situations provide signals (CS) of threat related to initial trauma (US). Most Veterans "learn" to extinguish the CR by distinguishing between CS and US. However, while extinction of the CR generally occurs for most combat Veterans, a significant minority of those exposed to combat develop PTSD (Kessler et al, 1995). The leading theory is that PTSD involves a failure of mechanisms involved in recovery and restitution of physiological homeostasis, possibly resulting from individual predisposition (Yehuda et al, 1995). These processes are collectively a failure of extinction of the stress response, and can be measured by subjective description, symptom reports, and biological testing.

Failure of Extinction in PTSD: Assessing with PPR. Physiological reactivity on exposure to CS is a core feature of PTSD. Research has demonstrated that individuals with PTSD have different physiological responses (e.g., startle, heart rate, skin conductance response) to CS compared to controls (Orr et al, 2000).

The startle response, measured with electromyography (EMG) of the eyeblink, provides an ideal translational tool to investigate fear conditioning and extinction, since the amygdala is directly connected with the startle circuit (Davis, 1992; Grillon et al, 1999). Fear-potentiated startle is the relative increase in the startle magnitude elicited in the presence of a CS; this PPR measure can be used to index both the increase in fear during conditioning as well as the reduction of fear during extinction. Traumatized individuals with PTSD show exaggerated startle compared to trauma controls (Jovanovic et al, 2010) during fear conditioning as well as during fear extinction (Norrholm et al, 2011c). Previous research indicates that impaired extinction is a good predictor of PTSD symptoms; specifically re-experiencing symptoms (Norrholm



et al, 2011c). In our previous studies, we have seen this impairment in two patterns. First, in two separate combat PTSD cohorts fear does not reach equivalently low levels in patients compared to controls, even after 6 blocks of extinction (Figure A) (Norrholm et al, 2011b). Second, in two independent civilian PTSD cohorts we observed a pattern of slower extinction rate (i.e., levels of fear in PTSD subjects are higher in early- and mid-extinction, but comparable to controls during late extinction) (Figure B).

Heart rate variability, heart rate, and skin conductance are good translational tools for assessing fear conditioning and extinction. While disturbances of both resting physiological tone and stress-reactivity in PTSD are established, stress-reactivity to CS is more pathognomonic in PTSD (reviewed by Orr) (Orr et al, 2000). Across various stimuli, skin conductance (SC) is the most sensitive measure of general arousal, whereas heart rate (HR) is more specific to trauma-related cues (Boucsein et al, 2012). The parasympathetic nervous system (PNS) influences basal HR and exerts a wider range of cardiac chronotropic control than the SNS branch in humans (Porges, 1995). PNS vagal influence on the heart can be evaluated by quantifying heart rate variability (HRV), measured as the amplitude of rhythmic fluctuations in HR associated with breathing (respiratory sinus arrhythmia [RSA]) (Sahar et al, 2001). Cohen and colleagues reported significantly lower resting RSA in PTSD than in controls and the absence of a HRV response to trauma recall (Cohen et al, 1998). Sack reported evidence of low RSA during exposure to trauma-related stimuli in 31 patients with PTSD (Sack et al, 2004). In 35 subjects who survived motor vehicle accidents who were monitored with 24-hour Holter ECG two days after the accident and evaluated for PTSD at 2 and 6 months after the accident, there was a global diminution of HRV associated with PTSD at both 2 and 6 months (Shaikh al arab et al, 2012). In another study, videotapes of varying emotional valence were presented to trauma-exposed participants with PTSD (n=26), trauma-exposed participants without PTSD (n=26), as well as non-trauma-exposed controls (n=18) while recording ECG and respiration. The PTSD group showed lower HRV than non-trauma-exposed controls at baseline (corrected for age) and throughout different affective conditions (Hauschildt et al, 2011). Recent work has shown that respiration rate and tidal volume contribute substantially to within-individual RSA variance (Schulz et al, 2009), and this group has developed a MATLAB toolbox for correcting within-individual effects of respiration rate and tidal volume on respiratory sinus arrhythmia during variable breathing, which we will adopt for this study.

Evidence for Extinction Learning in PTSD Patients. To support the use of PPR as outcomes, it is crucial to know if the PPR abnormalities might be reversed with treatment of PTSD.

A few published studies have reported PPR changes associated with treatment of PTSD. Orr and Roth reviewed a number of earlier studies (Keane & Kaloupek, 1982; Shalev et al, 1993; Boudewyns & Hyer, 1990) outlining the psychophysiological applications for assessing treatment outcome (Orr et al, 2000).

A meta-analysis reviewed literature about improvement in extinction learning in both humans and animals following exposure therapies (Norberg et al, 2008). While subjects in these studies did not have PTSD, they had other anxiety disorders (e.g., social phobia, panic disorder, and OCD). In each case, the efficacy of exposure treatment was associated with improved extinction learning. One study showed progressively lower arousal with treatment compared to sham therapy, evidenced by decreasing HR and increasing HRV across treatment (Sack et al, 2008). A more recent study showed low physiological responding during script-driven trauma imagery extended up to 4 months after treatment, showing the durability of treatment effect (Brunet et al, 2014).

PPR's during a conditioning paradigm have also been shown to predict treatment outcome in patients with PTSD (Aikins et al, 2011). Patients who were able to demonstrate differential conditioned fear responses at pre-treatment were more likely to display reductions in PTSD symptoms following duloxetine treatment, suggesting that conditionability is a potential biomarker for treatment outcome. As such, we may find that pre-treatment extinction learning is a predictor and/or a consequence of acupuncture treatment response. Jovanovic reviewed other conditioning studies related to PTSD, concluding that impaired safety signal learning and failure to extinguish conditioned responses are potential biomarkers for PTSD. The authors also call attention to the fact that future studies need to assess extinction learning both pre- and post-treatment to assess the efficacy of the treatment (Jovanovic et al, 2012). The studies reviewed only assessed extinction learning at baseline. The proposed research is thus a novel approach assessing biomarkers associated with PTSD and treatment outcome.

Acupuncture Has Effects on PTSD Biology and will Promote Extinction Learning

A significant literature suggests that acupuncture is likely to reverse PPR abnormalities seen in PTSD. There are no studies to our knowledge about biological effects of acupuncture in human PTSD. A recent review, however, describes a large human and animal literature about biological effects of acupuncture in systems involved in PTSD (Hollifield, 2011). Since we will assess ANS PPR's, we review information relevant to the ANS.

Neural pathways and autonomic (ANS) physiology (in (Hollifield, 2011). Effective acupuncture, which has been shown to occur when the sensation of "de qi" is obtained (Hui et al, 2005), stimulates α-delta fibers in the skin or muscle, which terminate in laminae I and V of the spinal cord. Marginal cells (M) in the cord project to somatosensory cortex via spinothalamic tracts, and projections to the medial prefrontal cortex (mPFC) travel by spinoreticular tracts, reticular formation (RF), and thalamus. Lamina I also projects to the locus coeruleus (LC), which is the adrenergic control center of the brain. In addition, downward projections via the fronto-arcuate connection to the hypothalamus extend to the descending inhibitory pathways directly to the LC and to serotonin and adrenergic systems. Manual acupuncture (MA) causes a broad CNS response involving the mPFC, amygdala, hippocampus, hypothalamus, cerebellum, basal ganglia, and insula, assessed by multiple imaging techniques, dependent on acupuncture type and frequency of stimulation (Napadow et al, 2005). The literature is replete with data about specific and broader, orchestrated effects of either MA or electro (EA) acupuncture on ANS, immune and inflammatory, and genetic expression via neural and protein messengers.

Acupuncture is generally sympathoinhibitory in animals (Middlekauff et al, 2001) and humans (Haker et al, 2000), although EA may cause either excitation or inhibition of the sympathoadrenal medullary reflex depending on EA location (Mori et al, 2000). A review by our senior advisor, who has conducted acupuncture research over the past two decades, details ANS physiology of acupuncture (Longhurst et al, 2013). Stimulation at known acupoints activates underlying sensory neural pathways that project to CNS regions that ultimately regulate ANS outflow. A long-loop pathway involving the hypothalamus, midbrain, and medulla underlies EA modulation of reflex increases in blood pressure (BP). Excitatory and inhibitory neurotransmitters in the supraspinal CNS underlie processing of somatic input and regulation of ANS outflow during EA. Acupuncture also decreases BP through actions in the thoracic spinal cord (Longhurst et al, 2013). Other studies have also demonstrated ANS regulation in clinical conditions. Studies in animal models of tachycardia (Wang et al, 2009) and depression (Zhou et al, 2007) have found changes in the expected direction of central and peripheral monoamine transmitters. Three human studies showed a change in serum catecholamines (CATS). One compared EA with the medication Nicardipine and found that both decreased systolic and diastolic blood pressure and CATS (Wan et al, 2009). One compared MA to the medication fluoxetine for post-menopausal symptoms and found that CATS change with both interventions similarly (Zhou et al, 2007). The third found that EA plus psychotherapy compared to psychotherapy alone in anxiety was more clinically effective and was associated with a greater reduction of CATS (Zhu et al, 2008).

Acupuncture and PPR. A growing literature about the effects of acupuncture on PPR is beginning to emerge, although these studies were not in human PTSD. For example, electrodermal activity (EDA) has been studied in relation to four commonly held tenets of acupuncture: 1) EDA at pathology-related acupuncture points are distinguishable from non-pathology points, 2) EDA measured at acupuncture points can assist in monitoring therapeutic progress, 3) EDA at acupuncture points can identify substances that are beneficial or toxic to an individual, and 4) acupuncture points have lower electrical resistance than the surrounding skin. Studies using auricular acupuncture for pain and drug abuse found that EDA was helpful to monitor therapeutic progress. A reduction of impedances and improved electrodermal balance along meridians during treatment corresponded with clinical improvement (Colbert et al, 2011).

Change in HR and HRV with acupuncture has been studied. An examination of differences in PPR to varying forms of MA showed that high-frequency and low amplitude manipulation led to decreased BP and HR, while low-frequency and high amplitude manipulation resulted in an initial increased BP and decreased HR and a more marked long-term decrease in BP (Backer et al, 2002). Chang et al. found a decrease in low frequency spectral power of HRV (related to sympathetic activation) associated with acupuncture points along the

pericardium meridian compared to sham points (Chang et al, 2008). Additionally, HRV power spectral analyses were utilized to demonstrate an increase in sympathetic activation during the first minute of the acupuncture session which included stimulation (likely triggered by pain or de qi), and an increase in parasympathetic power during the time following stimulation in verum compared to sham acupuncture (Streitberger et al, 2008).

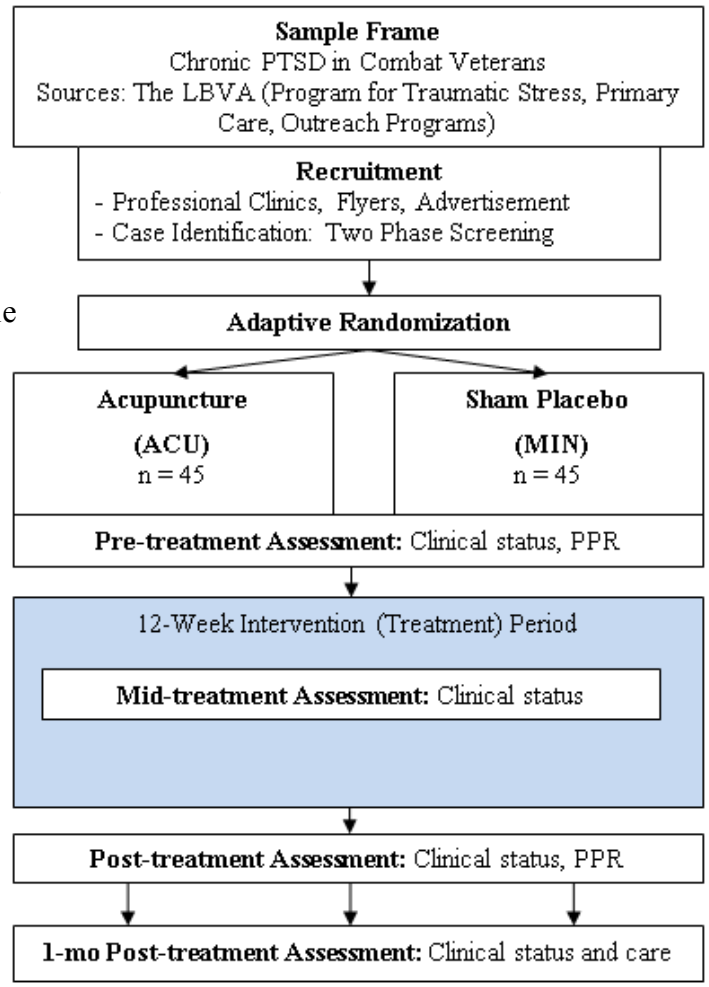
How does change in ANS PPR translate to improved extinction learning? The study is novel in examining the effects of acupuncture on extinction learning. Our theoretical framework posits that failure of extinction learning is central to PTSD pathology. We theorize that acupuncture will change sympathetic/parasympathetic balance, lessen the subjective experience of anxiety and generalized perception of danger, which will promote extinction to CRs. We will assess anxiety, PPR and extinction learning as evidence.

II. RESEARCH DESIGN & METHODS

Design and Protocol Summary

This is a 5-year study at the Long Beach VA Healthcare System (LBVA). Collaborating partners within the LBVA are The Program for Traumatic Stress' Novel Therapies Unit developed and directed by PI Michael Hollifield, MD; the Integrative Medicine Clinic founded and directed by Co-I An-Fu Hsiao, MD PhD; the Research Healthcare Group led by Chris Reist, MD MBA; and the PTSD psychophysiology laboratory managed by Mahmood Novin, MD. External collaborating partners include our design and analytic experts Besa Smith, PhD from UCSD and National University and Tyler Smith, PhD, Chair of Community Health at National University; the developers of our psychophysiology laboratory Tanja Jovanovic, PhD and Seth Norrholm, PhD from Emory University and the Atlanta VA.

Figure. Sample Frame and Flow of Project



Design. A two-arm, parallel-group, prospective randomized placebo controlled clinical trial to evaluate the efficacy of acupuncture for PTSD.

Study Timeline. Start-up and final IRB approvals will be completed by month 6. Subjects will be accrued at *2.5 per month* from study month 7 to 44. All subjects will complete treatment by month 47 and follow-up assessment by month 48. Analyses for primary hypothesis and all reports will be completed by month 57.

Participants and sampling. The sample frame is Veterans with chronic PTSD due to combat exposure seeking treatment at the LBVA or affiliated programs. The figure shows the flow of the project. Withdrawals prior to beginning intervention will be replaced. The sample size (90) provides adequate power to test primary hypothesis.

Inclusion criteria are meant to recruit a relatively homogeneous yet generalizable sample of Veterans with at least moderate chronic PTSD due to combat trauma. Criteria are: (1) Veterans aged 18 to 55, (2) DSM-5 criteria for chronic PTSD on the Clinician Administered PTSD Scale (CAPS-5), and (3) at least moderate PTSD by having a total CAPS-5 score of $\geq$ 26 and meeting criteria for each of 4 symptom clusters. Eligible persons will be allowed to have other symptoms that are commonly comorbid with PTSD (e.g., anxiety, mild to moderate depression), but these will not be inclusion or exclusion criteria. This strategy will provide a feasible and

generalizable sample of those with chronic PSTD. Women and minorities will be recruited.

Definition of "PTSD due to combat trauma:" Subjects must have PTSD due to a traumatic event in a combat zone. This may be any type of trauma, AND PSTD may be complex (i.e., combined war event, sexual event, MVA, etc).

We would ideally include an older age range of Veterans to increase sample frame and generalizability. However, autonomic nervous system (ANS) function (sympathetic/parasympathetic balance) begins to change at about age 55 (Pascualy et al, 1999; Shibasaki et al, 2013; Veith et al, 1988). Since acupuncture may work via the ANS, and since exploratory hypotheses are dependent on ANS functioning, and the ANS age-related changes are difficult to detect to rationally exclude subjects, the best choice is to exclude those older than 55.

Exclusion criteria are meant to keep out individuals with characteristics that are known to be PTSD treatment confounds, that may significantly affect biological assessment, that indicate past non-adherence or treatment resistance, or who may be put at risk of harm. Criteria are: (1) current and past six-months psychosis, (2) substance dependence (evidence of tolerance and/or withdrawal) within the past 6 months, (3) thyroid disease, (4) decisional incapacity (e.g., dementia), (5) centrally acting medications that have a potential effect on biological expression (e.g., beta-blockers, opiates, and ≥10mg equivalent of diazepam/day), (6) pain levels requiring opiate medications, (7) known exposure to chemicals or physical trauma that cause neuropsychiatric sequelae, (8) severe depression (Beck Depression Inventory-II score ≥30) that is deemed more clinically significant than PTSD, since this may bias accurate PTSD diagnosis and biological measures, (9) a diagnosed and untreated sleep breathing disorder (SBD) which is a treatment confound, (10) a high risk of a SBD as indicated by snoring ≥50 of nights plus one of (a) any witnessed apnea, (b) feeling non-refreshed in the morning ≥50 of mornings, or (c) daytime sleepiness indicated by falling asleep with routine tasks such as watching TV or reading, (11) non-response to ≥2 evidence-based PTSD treatments (adequate medication of 12 weeks or completion of PE, CPT or an intensive program), (12) treatment non-adherence indicated by stopping treatment or >3 missed appointments in the course of a PTSD EBT, (13) high dissociation as indicated by a score of ≥25 on the Dissociative Experiences Scale – II (Bernstein & Putnam, 1986), (14) past chronic PTSD prior to military service, (15) current active psychotherapy for PTSD, (16) having acupuncture in the past year, or (17) pregnancy. A person who is on a stable dose (8 weeks) of medication for depression, anxiety, PTSD, or for sleep, or any other psychoactive medication that may confound the study, and who meet entering criteria and will continue these medications for the duration of the trial will not be excluded (see Clinical Protocol Detail).

III. Procedures

Recruitment. A two-stage detection method will be utilized to identify a sample of PTSD treatment seekers. Based on our previous study, we expect a 15% drop-out rate prior to the start of intervention; these will be replaced. Subjects who withdraw after starting treatment will not be replaced: based on our previous study we expect a 10% rate. This would leave 40 in each intervention group for a treatment completion analysis. Data of subjects who withdraw at any time after randomization will be retained for intent-to-treat analyses.

Stage I: Screening for Inclusion/Exclusion. The PM/RA will contact potential subjects and ask them to provide written informed consent for study screening. Those who consent will be assessed with screening questions for inclusion and exclusion criteria, availability, and willingness to participate in the study. This visit may be conducted in person or by phone and will take about 20 minutes.

Stage II: Inclusion Confirmation. The assessor will administer The CAPS-5, a modified Structured Clinical Interview for Diagnosis DSM-5 (SCID-5, now modified) (First et al, 2001), and the Dissociative Experiences Scale-II (Bernstein & Putnam, 1985) to assess inclusion and exclusions and further assess willingness and capacity to participate (i.e., have time and motivation). The Deployment Risk and Resiliency Inventory (DRRI) combat experiences scale and preparedness scale (Vogt et al, 2008) will be administered to aid group allocation. The study PI will assess current medications as potential exclusions. This visit will take about 2 to 3 hours.

After confirmed inclusion, the PM will reiterate the schedule, group allocation and compensation, and obtain written informed consent for participation. This will include informed consent to be videotaped to assess treatment fidelity. All hard and electronic data forms will be identified only by SID#. The clinical assessor will assess participants at each time point blind to group allocation. This procedure will keep the assessor blind to intervention allocation, and investigators and clinicians blind to assessment data to minimize performance and outcome detection bias.

Randomization to intervention. The 90 subjects will be allocated to intervention group by a computer-generated adaptive randomization procedure. Because of the adequate but still modest sample size in this study, simple randomization may not provide group equality on variables that affect outcome. Adaptive randomization is better suited to provide group equality if there is some knowledge about what variables affect outcome. The technique of minimization, for example, is one type of adaptive randomization. While this technique may risk subversion or technical error (Hewitt et al, 2006), minimization has been shown to be the best method of ensuring excellent balance between groups for several prognostic factors in small to moderate samples (Scott et al, 2002; Treasure et al, 1998), where blocking and stratification are not effective in small trials. With minimization the treatment allocated to the next participant enrolled in the trial depends on the characteristics of those participants already enrolled to minimize the imbalance across multiple factors. Minimization aims to ensure treatment arms are balanced with respect to predefined patient factors as well as for the number of patients in each group. Simulation studies show that minimization provides better balanced treatment groups when compared to randomization methods such as permuted blocks within strata (Scott et al, 2002). While there is still very little work about factors that predict outcome in PTSD, one study in Veterans showed that combat exposure and pre-deployment preparedness accounted for significant outcome variance using standard exposure-based psychotherapy (Price et al, 2013). There are also data suggesting gender effects of emotional disclosure interventions for PTSD in non-Veteran subjects (Ironson et al, 2013). As such, allocation to group will use an adaptive randomization designed to provide group equality in descending priority on: (1) number in each group, (2) combat exposure, (3) pre-deployment preparedness, and (4) gender. This will be accomplished by using a software program – minim – that will be adapted for the study by the PI and Dr's. Smith. One potential problem of adaptive randomization is added organizational complexity and possible subversion of the randomization. Dr. Besa Smith will work with the DA to ensure that the program is clear and easy to use, and they will "sample enroll" 20 Veterans from our program prior to the study to demonstrate ease and effectiveness. The risk of subversion will be controlled by having only one person (DA) manage the minim program and discuss ongoing enrollment statistics with only the statisticians.

The DA will assign each subject a Treatment ID number (e.g., TID# A1 to A45 for ACU) and consecutive study ID numbers (i.e., SID# 1 - 90), which will be the only number on hard copies of documents to conceal allocation from all investigators and other staff. The DA will be the only study staff that will be able to link both SID and TID numbers to subject and will be solely responsible for providing the clinician with treatment allocation. Other study personnel will be blind to this process and will only see the SID#.

Interventions. Group 1 will receive ACU delivered in 1-hour sessions, twice per week for 12 weeks, Group 2 (placebo control), will receive MIN, also delivered in 1-hour sessions, twice a week for 12 weeks. (See Clinical Protocol Detail).

Subjects in both groups will receive an equivalent amount and kind of time, empathy, setting, and assessment. Both verum and control protocols will include language currently in our acupuncture clinical trials manual for greeting, interacting with, and closing a session.

Informing subjects about group allocation. MIN acupuncture is indistinguishable from verum ACU to subjects who have not received this intervention and to most people independent of acupuncture experience. However, blinding may be compromised unless care is taken during allocation, informed consent, and the actual sessions. Clinical Protocol Detail has a more complete description about how study procedures inform subjects about group allocation in a way that maintains integrity of blinding to intervention. These procedures include how the room and materials are set up, how interventions are discussed, and how informed consent is delivered.

Group 1: Verum acupuncture (ACU). Individual treatment sessions are 1 hour twice per week for 12 weeks, and reflect clinical practice with an interview (10 minutes), pulse and tongue observation (5 minutes), standard needling, and needle retention (30 minutes). Subjects receive a standard acupuncture point prescription defined in our previous study and chosen for the most likely TCM diagnostic patterns for PTSD. An alternating by session front and back treatment will be used to avoid point fatigue (tolerance due to frequent use). The front treatment is comprised of 11 needles, bilaterally at LV3, PC6, HT7, ST36, SP6, and at the single Yintang point; the back treatment is 14 needles, bilaterally at GB20, and UB14, 15, 18, 20, 21, and 23. In addition to the standard points, three additional points (chosen from a list of 15 points) will be chosen to address a subject's constitution based on the TCM diagnostic patterns. A complete description of the experimental intervention is provided in published reports (Hollifield et al, 2007; Sinclair-Lian et al, 2006) (PMID: 17568299 and 16494568).

Group 2: Sham acupuncture: minimal needling (MIN). Individual treatment sessions are 1 hour twice per week for 12 weeks. Three elements define minimal needling in this study. The first is the location of the needles, which are 2 cm lateral or medial to actual points, which are not associated with PTSD (Sinclair-Lian et al, 2006) and are not expected to effect PTSD symptoms. The second is the insertion depth, which will be superficial (<0.25 inch) in MIN compared to verum. The third is the relative absence of stimulation, although there will be a non-functioning stimulator used to complete the sham effect. The acupuncturist will ensure that no DeQi sensation is obtained. The protocol uses 11 front and 14 back points for the sham acupuncture group (MIN) to match the number and body position (alternating prone and supine) of points in the verum acupuncture group (ACU).

Compensation Plan. Subjects will be compensated for their time and travel to participate in the study. Subjects will be compensated $25 each for initial and post-treatment assessment, $50 for each of 3 biological assessments, and $50 for completing the intervention for a total possible compensation of $250 per subject.

Protection of Human Subjects: See Human Subjects Detail

III. Data Management

Data collection. The program manager (PM) will track recruitment and retention. A clinical assessor will conduct diagnostic interviews and questionnaire evaluations pre-, mid-, and post-intervention blind to group allocation. All pre-, mid-, and post-treatment biological measures will be conducted and analyzed by the psychophysiologist and consultants, blind to group allocation.

Assessment, Data Collection, and Schedule. Instruments and other data collection tools and their description are in the Assessment Detail. This strategy includes repeat measures yet minimizes response burden. Our goal is to test for effect signal. It is premature to evaluate durability and not ethical to keep subjects in a protocol where they may not be receiving effective treatment (particularly true of MIN), so we will not include a six-month follow-up period. Instead, a one-month follow-up will test symptoms and functioning and be utilized to re-connect participants to clinical services. Data collection at mid-point of treatment is warranted to replicate mid-treatment findings from our first trial. Such a finding may help determine dose effects and plan a dose-finding trial.

Outcome measures. The primary outcome is change in PTSD symptom severity from pre- to post-treatment. We hypothesize a large pre- to post-treatment effect for ACU ($d \geq 0.8$) and at least a mild treatment effect ($d \geq 0.30$) of the difference between groups (ACU vs MIN) pre- to post-treatment, with 80% probability of detecting a true group difference at $p<0.05$ (2-sided). Main biological outcome (secondary outcome) is change from pre- to post-treatment in PPR (decreased startle by EMG eyeblink).

IV. Statistical Plan and Data Analyses

General Approach. General linear mixed models (GLMM) are capable of handling multiple underlying distribution and model structures through link functions, such as repeated measures random effects models of continuous outcomes (identity link), repeated measures logistic models (logit link), and Poisson and negative binomial models (log link). In addition to modeling global fixed effects across subjects, GLMM can also model individual subject random effects. Survival analysis allows for the assessment of time to event modeling using probability estimates on survivor functions and estimates of hazard ratios. Time to event modeling using Cox proportional hazard modeling allows for all subjects to contribute information to the model up until occurrence of event, drop out, or end of study. Cox Proportional Hazards modeling will be used to investigate for effect-size of the treatment by allowing subjects to contribute their information to the model while they are being observed and censored once they are no longer being observed (loss to follow-up or end of study period). Further, using the Cox modeling we will investigate for time dependencies and informative loss to follow-up. In the case where dropouts may be associated with the treatment assignments, we will leverage intention to treat methodology and construct a piecewise random effects model with both "on-" and "off-treatment" slopes. GLMM will be used to evaluate the primary clinical hypothesized effects of treatment (ACU) on the clinical outcome of PTSD symptom severity (CAPS) over time (mid- and end-treatment, and 1-month follow-up), controlling for baseline severity of symptoms and demographic characteristics (e.g., age, gender) in comparison with placebo control group (MIN) with assumption of intent to treat. Cohen's d within and between subjects will be calculated. Interaction terms will be included in the models to evaluate treatment fidelity and treatment expectancy as potential moderators.

GLMM will also be used to evaluate the secondary biological hypothesized effects of treatment on pre- to post-treatment in PPR (decreased EMG eyeblink). These same statistical procedures (GLMM, survival analysis, and Cohen's *d* with intent to treat) will be applied to evaluate exploratory outcomes: clinical symptoms comorbid with PSTD, PPR (HR, HRV, SCR), and PTSD diagnosis.

Statistical Analyses, Primary Hypothesis. Prior to the application of any statistical modeling, underlying assumptions and conditions will be examined. Univariate analyses, including tests of distribution assumptions, t-tests, and chi-square tests will be conducted to determine possible significant covariates to be included in further multivariable modeling as well as to investigate efficiency of randomization techniques. All models will be assessed for goodness of fit as well as other diagnostics including assessment for collinearity performed on all covariates chosen for subsequent analyses. Repeated measures analysis of variance will be used to formally test the null hypothesis of no differences in baseline PTSD symptom mean levels and subsequent retest of PTSD symptom mean levels while simultaneously adjusting for any significant covariates. All models will be run accounting for any potential confounders. Analyses will be performed assuming intent to treat and informative loss-to-follow up will be investigated. Additional analyses will be conducted to determine interactions and mediating effects while simultaneously adjusting for other covariates in the model.

Assumptions for Power Calculations. Assumptions are based on interpolated data since primary outcomes in our first ACU study were assessed with the PSS-SR scale and there are no PTSD studies using MIN as the sham. Furthermore, to be consistent with major PTSD research, we will use the CAPS-5 (DSM-5 version) as the primary outcome, and there are as of yet no completed clinical trials using CAPS-5. In our first study, mean pre-treatment PTSD severity score was about 62% of maximum (31.55 of 51). ACU mean decreased 50% - 31.33 (10.1) to 15.65 (13.95) and wait-list mean decreased 9.3% - 30.79 (9.54) to 27.92 (12.33) at post-treatment. We thus assume that subjects will have a baseline mean CAPS-5 score of 50 (10) and a 50% reduction of symptoms and a 38% increased variance (CAPS-5 = 25; SD = 13.8) with ACU. There are sparse data about effects of MIN, noting a 33% improvement compared to pharmacological treatment only, and a 33% less effect than ACU in the Shen study. A 2010 Cochrane systematic review of over 200 trials investigating 60 clinical conditions about general placebo effect found placebos to not have important clinical effects but may influence patient-reported outcomes in some situations (e.g., pain and nausea). The pooled relative risk calculated for placebo was 0.93 (effect of only 7%) but significant. Confidence intervals are generally wide in the placebo arm. Several clinical and methodological factors were associated with higher effects of placebo. Since our study

includes some of these factors, and since we expect MIN to have some physical effect, MIN could provide an effect as much as 33% with a wider variance. This would predict mean CAPS-5 scores (MIN) at end-treatment of 33.5 (SD 15). The conservative prediction is mean CAPS-5 reductions of 25 points with ACU and 16.5 points with MIN, an 8.5-point difference with a pooled SD of 14.4. Given our experience and data about placebo effect, we can modestly expect a 12 point between group CAPS-5 difference and a pooled SD of 15.

Treatment Effects, Power and Sample Size, Primary Hypothesis. "The efficacy of verum acupuncture (ACU) for PTSD symptom severity will be large (pre- to post-treatment Cohen's $d \geq 0.8$), and significantly better than sham acupuncture (MIN) (between group Cohen's $d \geq 0.30$, with 80% probability of detecting a true group difference at $p<0.05$ (2-sided)."

Effects. The conservative assumptions noted above will result in pre- to post-treatment Cohen's $d = 2.07$ within group (ACU) and between group (post-treatment difference) Cohen's $d = 0.59$, which will prove the null hypothesis false and show a large treatment effect for ACU and a moderate between-group effect size.

Sample size needed and power. The conservative assumptions would require a total of 90 patients in a two-treatment parallel-design to provide a probability of 80 percent to detect a treatment difference at a two-sided 0.05 significance level. Modest assumptions of a 12-point difference with a pooled SD of 15 requires a total of 50 patients with power of 0.80 at alpha <0.05.

This was determined using a formula for a random effects modeling (Montgomery, 1991), which may determine the necessary sample size to detect a statistically significant difference in treatment/control group means at different levels of projected effect differences.

$$n = \frac{2*a*M^2*\Phi^{*2}}{\Delta^2}$$

Where:
M = measure of noncentrality parameter to estimate the standard normal probability of making a type II error
n = number of replicates necessary for each treatment
a = number of treatments (a=2)
Φ^* = estimated standard deviation
Δ = difference in baseline and post treatment

Table. Examples of sample sizes necessary for significance testing at different mean and SD's (between group: treatment (ACU) and control (MIN)) for PTSD severity (primary outcome) at power = 0.80.

Mean difference between ACU and MIN PTSD severity scores post-treatment	Φ	Total N Using Formula Above
8.5	12	62
	14.4	90
	15	96
	20	162
12	12	32
	15	50
	20	86

Recruitment goals are N=90, 45 per group. With a post-randomization attrition rate of 10% we will have at least 40 individuals in each group. Considering attrition, the above table indicates that for an average difference in means of 12 at end-treatment with a standard deviation of 15, a total sample size of 50 (25 in each group) would be more than adequate to report a statistical difference with 80% power. If differences in treatment group means were found to be larger than 12, we would need even fewer subjects in our treatment groups to achieve statistical significance.

PPR Data Recording and Reduction. A description of the procedure/data is in Appendix 4. Data reduction and analyses will follow current established protocols in our lab (Norrholm et al, 2011a). Data will be collected with the BioSemi recording software (BioSemi B.V, Amsterdam, Netherlands) and the resulting data will be exported to Mindware software (Mindware Technologies LTD, Gahanna, OH) for data reduction and generation of analyzable variables.

Startle data reduction for analyses. The raw EMG signal will be recorded at a rate of 1000 Hz throughout the experimental session using a 28 Hz high pass and 500 Hz low pass filter (as recommended by guidelines for human eyeblink startle in Blumenthal et al., 2005; Psychophysiology, 42:1-15). Raw signals will be stored and exported for analysis in microvolt (μV) values.

Skin Conductance Response. Skin conductance responses are scored as the largest amplitude responses beginning in a window of 1 to 3 seconds following stimulus onset. A response is defined as having amplitude greater than 0.01 μS relative to the pre-stimulus baseline (Boucsein et al, 2012).

Heart rate. Average heart rate (HR), the standard deviation of the HR, power of high frequency (HF), low frequency (LF), very low frequency (VLF) components, % power of LF (%LF [of VLF+LF+HF]) and of HF (%) and the ratio of the LF over the HF (LF/HF ratio is used as an indirect autonomic balance index) of HRV are calculated as cardiac activity measures. Artifact-corrected 3-min long recording epochs are analyzed with FFT to assess HRV. Inter-beat Intervals (IBIs) will be scored as the time difference between successive R waves in the ECG signal. IBIs will be used as the dependent variable analyzed instead of heart rate because of a lowered susceptibility to artifact due to differences in baseline values (Stern et al., 2001). A window of 3 seconds pre-stimulus onset to 6 following stimulus onset will be scored. Instantaneous IBIs will be recorded at half-second intervals during the pre- and post-stimulus time windows. A difference score between the average pre-stimulus IBI for each trial and each post-stimulus IBI value will be computed for each trial. IBIs will later be converted heart rate (beats per minute) for more readily interpretable post-analytic write-ups.

Respiration. Respiration will be scored in a similar fashion to the ECG data and reported in interbreath intervals. Peak detection of each positive deflecting curve in the breathing cycle will be manually reviewed visually to assess accuracy. Interbreath intervals will later be converted to respiration rate (breaths per minute) for ease of interpretation.

Clinical Protocol Detail

Interventions. All subjects will be allocated to intervention group by a computer-generated adaptive randomization procedure. Selected participants will go through a preliminary phase to assess if there are specific acupuncture points that change heart rate and heart rate variability more than others during the psychophysiology assessment.

Group 1 will receive verum acupuncture (ACU) (experimental intervention) delivered in 1-hour sessions, twice per week for 12 weeks.

Group 2 will receive minimal acupuncture (MIN) (sham placebo control), also delivered in 1-hour sessions, twice a week for 12 weeks.

Subjects will be blinded to intervention (i.e., if experimental or sham). Clinical assessor(s) and investigators will be blinded to randomization. The acupuncturist cannot be blinded, and so will follow a standardized protocol to minimize intervention bias and treatment fidelity will be assessed.

Maintaining Blind and Non-specific Treatment-related Effects

Informing subjects and maintaining blind about group allocation. MIN acupuncture is indistinguishable from verum ACU to subjects who have not received this intervention and even to most people independent of acupuncture experience. However, blinding may be compromised unless care is taken during allocation, informed consent, and the actual sessions. McManus and colleagues have described practical steps to take before, during, and after each session to not compromise subject blinding using verum versus sham acupuncture (McManus et al, 2007). We will adopt these steps, which include how to set up the room and materials prior to treatment, the materials to have on hand, and how to insert, manipulate, and remove the needles. McManus utilized these procedures in an RCT (N = 135), providing 8 sessions over 4 weeks using about 10 needles per session and were successful by having 71% of those receiving sham and 81% of those receiving verum ($p = 0.20$) believe they received "real" treatment. Another important procedure for maintaining subject blinding is the construction and delivery of the informed consent. Subjects will all be informed that they will receive the standard dose of paroxetine, which is FDA approved for PTSD, and that there are "two types of treatments that involve acupuncture needles" or "two forms of treatment with needles" that will be used during this clinical trial, and that, they will be randomly allocated to receive either one or the other treatment. They will not be told the name or type of each protocol. If they ask about our expectation of effects, they will be told that we do have predictions, but that we cannot share with them those predictions. Any questions they ask about the needles or technique will be responded to with a structured answer that will be part of the training of all staff prior to the trial, such as, "as mentioned in the consent, there are two types of acupuncture treatments being used, and you may receive either one during any given session." This approach is necessary to ensure the best blinding for this trial and is ethical since the evidence is not yet available to say that either verum or sham is known to be superior treatment for PTSD.

Controlling for non-specific treatment-related effects. In this study, it is critical for those in the control group (MIN) to receive an equivalent amount and kind of time, empathy, setting, and assessment as those in the verum acupuncture group (ACU). Both verum and control protocols will include language currently in our acupuncture clinical trials manual for greeting, interacting with, and closing a session. Prior to the first session, each subject will meet with the acupuncturist who will conduct a Traditional Chinese Medicine (TCM) diagnostic session. A protocol example from our first acupuncture for PTSD study is Appendix A to this Attachment and included pragmatics and acupuncturist behavior. Note that this example differs in content from the proposed study. On funding, a protocol manual will be developed.

Outline of Steps of Procedures and Interventions

Step one: Conduct informed consent and conduct pre-treatment evaluation

Step two: Randomization to group

Subjects will be allocated to group by adaptive randomization.

Step three: Begin and continue ACU or MIN interventions

Step four: After 12-week intervention, stop ACU and MIN and conduct End-Treatment Assessments

Step six: At the end of follow-up period (planned one month), determine how and where to transition subject to clinical care

Note: One task of Data Safety and Monitoring on funding will be to review the protocol and advise the investigators about rules for continuation or discontinuation of each subject in the study and in the follow-up period. It may be unethical to continue Veterans in the follow-up period if there is no pre- to post-treatment reduction in CAPS. Furthermore, we are most interested in whether there is effect maintenance in those who have responded to treatment, so it may be scientifically meaningless in addition to the ethical dilemma to continue non-responders into the follow-up period. In this A1 revision, our primary goal is to determine if ACU provided effect (signal) compared to a placebo, and the follow-up period is not to primarily determine durability of treatment but to ensure safety and clinical follow-up planning. Our statistical methods will be able to manage data in an intent-to-treat model if subjects are withdrawn. Our current plan is to define a response as a CAPS-IV equivalent drop of 10 points (the value for CAPS-5 still needs to be determined). Those who have not had a response would have the option to withdraw and re-initiate clinical treatment immediately. Of course, any subject may withdraw at any time.

Description of Intervention Protocols

Recruitment of participants currently on medications for PTSD. It may be ideal to recruit subjects who are medication naïve, but this is not feasible in this single site study. Approximately 55% of Veterans with PTSD who are entering three of our current studies (a DoD funded multi-site trial of prolonged exposure with vs without virtual reality, Diefede PI, Reist, Long Beach site PI; a VA national cooperative studies program comparing prolonged exposure to cognitive processing therapy – CSP #591, Schnurr et al PI's, Hollifield Long Beach site PI and executive committee member; an industry sponsored two-site RCT of Vilazodone vs. placebo for PTSD, Hollifield and Ramaswamy Co-PI's) are on a medication when entering the trial, most often an SSRI. In the first two mentioned studies, the subjects can stay on the medication (with rules), and for the third study we offer a wash-out if the current medication has not been established for a long period or if effects are neutral or negative. We will thus offer a similar procedure in the proposed study to the first two mentioned studies, and the subject must be on a stable dose for 8 weeks or longer.

Verum acupuncture (ACU). Individual treatment sessions are 1 hour twice per week for 12 weeks, and reflect clinical practice with an interview (10 minutes), pulse and tongue observation (5 minutes), standard needling, and needle retention (30 minutes). Subjects receive a standard acupuncture point prescription defined in our previous study and chosen for the most likely TCM diagnostic patterns for DSM-IV PTSD: Liver Qi stagnation (LV Qi); Heart Shen disturbance (HT Shen), and Kidney deficiency. An alternating by session front and back treatment will be used to avoid point fatigue (tolerance due to frequent use). The front treatment is comprised of 11 needles, bilaterally at LV3, PC6, HT7, ST36, SP6, and at the single Yintang point; the back treatment is 14 needles, bilaterally at GB20, and UB14, 15, 18, 20, 21, and 23. In addition to the standard points, three additional points (chosen from a list of 15 points) will be chosen to address a subject's constitution based on the TCM diagnostic patterns. The full protocol and the outcome of this protocol in PTSD was empirically developed and tested in our previous study, and is in the published papers (Hollifield et al, 2007; Sinclair-Lian et al, 2006) (PMID: 17568299 and 16494568), which may be found in Attachment 2. Table 1 below shows the standard front and back points for the primary patterns that are used for each subject and the possible additional points for the secondary patterns that are determined by TCM diagnosis.

Table 1. Acupuncture Points for The Treatment of Posttraumatic Stress Disorder				
Primary Patterns (Standard points for all subjects)				
	HT Shen Disturbance	**LV Qi Stagnation**	**Kidney Deficiency**	**Grounding points / Qi & Blood Deficiency**
Front points	HT7, (PC6) and Yintang (even)	LV3, PC6 (even)		ST36, SP6 (even)
Back points	UB14, 15 (even)	GB 20, UB18 (even)	UB 23 (reinforce)	UB 20, 21 (even)

Secondary Patterns (Up to three points chosen)						
	LV overacting on SP	**LV overacting on ST**	**ST Fire**	**LV Fire**	**Phlegm-Heat**	**Phlegm-Damp**
Front points	LV13 (reinforce)	LV14 (reduce)	ST44 (reduce)	LV2 (reduce)	ST40 (reduce)	SP9 (reduce)
Back points	UB18 (reduce) UB20 (reinforce)	UB18 (reduce) UB21(reduce)	Du 14 (reduce) UB21 (reduce)	Du 14 (reduce) UB18 (reduce)	Du 14 (reduce) UB21 (reduce)	UB20 (reduce)

	HT Yin/Blood deficiency	**SP Qi/Yang deficiency**	**KI Yin/Essence deficiency**	**KI Yang/Qi deficiency**	**LV Yin/Blood deficiency**	**ST Yin deficiency**
Front points	HT6 (reinforce)	SP3 (reinforce)	KI6 (reinforce)	KI7 (reinforce)	LV8 (reinforce)	ST44 (reinforce)
Back points	UB17 (reinforce) UB15 (reinforce)	UB20 (reinforce) UB23 (reinforce)	UB52 (reinforce) UB23 (reinforce)	Du4 (reinforce) UB23 (reinforce)	UB17 (reinforce) UB18 (reinforce)	UB21 (reinforce)

Disposable, stainless acupuncture needles (34-gauge Cloud Dragon) are inserted perpendicularly to a standard depth (from ¼" to 1½"). For subjects who are pain sensitive, Seirin needles maybe use because they are 40-gauge and better tolerated. Acupuncture needles are inserted with an introducer and are manually manipulated with insertion with the goal of obtaining "de Qi." The acupuncturist feels sensation from needle manipulation and the patient feels soreness or fullness but not sharp pain. After de Qi is achieved, microalligator clips and electrodes to a battery-operated pulse generator connected to the negative pole will be attached to needles at HT7 and ST36, and microalligator clips and electrodes connected to the positive pole will be attached to needles at PC 6 and SP6 (pairs HT7 – PC6 and ST36 – SP6). When the subject receives the back points, the electrodes will be attached to UB15 and UB20 (neg) and UB 14 and UB21 (pos), respectively (pairs UB15 – UB 14 and UB20 – UB21). An EA stimulator made by Pantheon Research (Pantheon 12-Pro) will be used because this stimulator has high fidelity in delivering electrical output. Mixed electrical frequency (alternating between 2 Hz and 100 Hz) will be used because prior studies have shown that it has greater impact on ANS and CNS activities compared with fixed electrical frequency (Han et al., 2013). Subjects will be told that they may or may not feel the stimulation. After 15 minutes, the clinician will enter the room and manually manipulate the needles that do not have electrical stimulation to obtain de Qi. After 30 minutes of needle retention the clinician

will enter the room, manipulate the needles that do not have electrical stimulation to obtain de Qi, and then remove needles in a prescribed order after turning off electrical stimulation.

Sham acupuncture (MIN). Individual treatment sessions are 1 hour twice per week for 12 weeks. Three elements define minimal needling in this study. The first is the location of the needles, which will be non-acupuncture points not near points found in our development work to be associated with PTSD (Sinclair-Lian et al, 2006) and are points not expected to effect PTSD symptoms. The second is the insertion depth, which will be superficial (<0.25 inch) in MIN compared to verum ACU. The third is the relative absence of stimulation. There will be a non-functioning stimulator used to complete the sham effect. The acupuncturist will ensure that no DeQi sensation is obtained. The protocol uses 11 front and 14 back points for the sham acupuncture group (MIN) to match the number and body position (alternating prone and supine) of acupoints in the verum acupuncture group (ACU). Insertion will be 2 cm medial or lateral to the actual defined acupoints. The 11 front points will be near: bilateral LU7, bilateral LU6, bilateral SJ3, bilateral SI4, bilateral ST 34, and left SI 19. Microalligator clips will be attached to needles at LU6 (positive pole) connected with SJ3 (negative pole), and between SI4 (positive) and SI 19 (negative). The 14 back points will be near the following bilateral points: SI 9, SI 11, SI 15, GB 30, GB 32, GB 33, GB 34. Microalligator clips will be attached between SI 9 (positive) and SI 14 (negative) and between GB 32 (positive) and GB 34 (negative). The microalligator wires will be connected to an electrical stimulator that is identical to and will deliver the same audiovisual stimuli as the one used for ACU; however, *no electrical current* will be passed through needles (Pantheon Research can modify their stimulator to maintaining the flashing green light without electrical current to be a pseudo-stimulator). Subjects in both ACU and MIN groups will receive identical time and attention in interview, pulse and tongue observation, and needle retention. Subjects will be told that they may or may not feel the stimulation. After 15 minutes, the clinician will enter the room and touch but not manipulate the needles that do not have pseudo-electrical stimulation. After 30 minutes of needle retention the clinician will enter the room, manipulate the needles that do not have pseudo-electrical stimulation, and then remove needles in a prescribed order after "turning off" pseudo-electrical stimulation.

Justification for choosing minimal needling as sham control. Sham procedures are not fully inactive and indistinguishable from verum acupuncture (Park et al, 2002). There are essentially 3 ways to conduct sham acupuncture using needles. Two involve needle insertion: (1) insertion and manipulation in the same manner but at purportedly "irrelevant" points, usually a few millimeters from actual verum points, or (2) superficial insertion at verum points, usually without manipulation to not elicit the "de qi" sensation. Each, or a combination of each, have been labelled "minimal needling." Investigators have also used various gauge-size needles and very small monofilaments as insertive shams. The third type of sham (3) is non-insertive, where a blunt needle within an adhesive O-ring mechanism hits the skin and retracts without puncture. When this sham is used, the needles for verum acupuncture are also administered via the adhesive O-ring for blinding. Both insertive and non-insertive procedures probably influence expectation, sensation, and contextualization, and both have shown effects in brain areas controlling sensation, cognition, and affect (Kong et al, 2006). While two non-insertive needles have been developed and shown to be valid shams from the subjective patient view (the "Streitberger" and the "Park Sham Device") (Park et al, 2002; Schnyer et al, 2008), these are far more cumbersome and very expensive compared to minimal needling. And, research has shown clinical differences between verum ACU and minimal (MIN) needling techniques, for example in a study of chemotherapy-induced emesis control (Shen et al, 2000) and blood pressure control (Flachskampf et al, 2007). In the Shen study where all patients had basic pharmacotherapy for emesis, emesis events and emesis free days were significantly better with ACU vs. MIN vs. pharmacotherapy only (events 5 vs. 10 vs. 15, respectively; free days 55% vs. 29% vs. 20%, respectively). MIN is the best available sham control to verum ACU because they both are similar in all aspects except that MIN includes irrelevant acupoints and shallow insertion without obtaining DeQi.

Psychoactive Medications Requiring 8 weeks stability

Drug Classes	Generic Names	Trade Names
OPIOID ANALGESICS	ACETAMINOPHEN/ HYDROCODONE	VICODIN
	ALFENTANIL HCL	AFLENTA
	BELLADONNA/OPIUM	BELLADONNA AND OPIUM
	BUPRENORPHINE	BUPRENEX/SUBUTEX
	BUPRENORPHINE/ NALOXONE	SUBONOXONE
	BUTORPHANOL TARTRATE	STADOL
	CODEINE	CODEINE
	CODEINE/ ACETAMINOPHEN	TYLENOL-CODEINE
	METHADONE	DOLOPHINE, METHADOSE, DISKETS
	MORPHINE	DURAMORPH, AVINZA, ASTRAMORPH, DEPODUR
	OXYCODONE HCL	OXYCONTIN
	REMIFENTANIL	ULTIVA
	SUFENTANIL CITRATE	SULFENTA
OPIOID ANTAGONIST ANALGESICS	NALTREXONE HCL	VIVITROL, REVIA
NON-OPIOID ANALGESICS	ACETAMINOPHEN/ BUTALBITAL/ CAFFEINE	ALAGESIC, ANOLOR, DOLGIC, DOLMAR, ESGIC,EZOL, FIORCET, GEONE, MARGESIC, MEDIGESIC, NONBAC, ORVIVAN, PERCAPS, REPAN, ZEBUTAL
	ASPIRIN/BUTALBITAL/ CAFFEINE	FIORINAL, BUTALBITAL COMPOUND, FARBITAL, FIORTAL
	CLONIDINE HCL	DURACLON
	TRAMADOL HCL	ULTRAM, RYZOLT
ANTIMIGRAINE AGENTS	CAFFEINE/ ERGOTAMINE	CAFERGOT, WIGRAINE
	DIHYDROERGOTAMIN E MESYLATE Spray	MIGRANAL
	SUMATRIPTAN SUCCINATE	IMITREX
BARBITURIC ACID DERIVATIVE SEDATIVES/ HYPNOTICS	PHENOBARBITAL	PHENOBARBITAL

BENZODIAZ DERIVATIVE SEDATIVES/ HYPNOTICS	ALPRAZOLAM	XANAX
	CHLORDIAZEPOXIDE HCL	LIBRIM
	DIAZEPAM	VALIUM
	LORAZEPAM	ATIVAN
	MIDAZOLAM HCL	VERSED
	OXAZEPAM	SERAX
	QUAZEPAM	DORAL
	ESTAZOLAM	PROSOM
	CLONAZEPAM	KLONIPEN
	FLURAZEPAM	DALMANE
	TEMAZEPAM	RESTORIL
	TRIAZOLAM	HALCION
SEDATIVES/ HYPNOTICS, OTHER	BUSPIRONE HCL	BUSPAR
	CHLORAL HYDRATE	NOCTEC
	ZOLPIDEM	AMBIEN
	ZALEPLON	SONATA
	HYDROXYZINE	ATARAX, VISTARIL
	ZOPICLONE	IMOVANE
	ESZOPICLONE	LUNESTA
ANTI-CONVULSANTS	CARBAMAZEPINE	TEGRETOL, CARBATOL, EQETRO
	DIVALPROEX	DEPAKOTE
	FELBAMATE	FELBATOL
	GABAPENTIN	NEURONTIN, GRALISE
	LAMOTRIGINE	LAMICATAL, LAMICTAL XR
	LEVETIRACETAM	KEPPRA, KEPPRA XR
	OXCARBAZEPINE	TRILEPTAL, OXTELLAR XR
	PHENYTOIN	DILANTIN, DILANTIN 125, DILANTIN KAPSEALS
	PRIMIDONE	MYSOLINE
	TOPIRAMATE	TOPAMAX
	VALPROIC ACID	DEPAKENE, STAZOR
	ZONISAMIDE	ZONEGRAN
ANTI-PARKINSON AGENTS	APOMORPHINE	APOKYN
	CARBIDOPA /LEVODOPA	SINEMET, SINEMET CR
	ENTACAPONE	COMTAN

	RASAGILINE	AZILECT
	ROPINIROLE	REQUIP, REQUIP XL
TRICYCLIC ANTIDEPRESSANTS	AMITRIPTYLINE HCL	ELAVIL
	CLOMIPRAMINE	ANAFRANIL
	DESIPRAMINE HCL	NORPRAMIN
	DOXEPIN HCL	PRUDOXIN, SINEQUAN, ZONOLAN, SILENOR
	IMIPRAMINE HCL	TOFRANIL, TOFRANIL-PM
	NORTRIPTYLINE	PAMELOR
	AMOXAPINE	AMOXAPINE
	TRIMIPRAMINE	SURMONTIL
	PROTIPTYLINE	VIVACTIL
MONAMINE OXIDASE INHIBITOR ANTIDEPRESSANTS	PHENELZINE SULFATE	NARDIL
	SELEGILINE	ELDEPRYL, ZELPAR
	TRANYLCYPROMINE SULFATE	PARNATE
ANTIDEPRESSANTS ,OTHER	BUPROPION HCL	WELLBUTRIN
	CITALOPRAM HYDROBROMIDE	CELEXA
	ESCITALOPRAM	LEXAPRO
	FLUOXETINE	PROZAC
	MIRTAZAPINE	REMERON
	PAROXETINE	PAXIL
	SERTRALINE	ZOLOFT
	TRAZODONE	DESYREL
	VENLAFAXINE	EFFEXOR
	NEFAZADONE	SERZONE
	DULOXETINE	CYMBALTA
	MILNACIPRAN	SAVELLA
	LEVONMILNACIPRAN	FETIZMA
	VENLAFAXINE	EFFEXOR
	DESVENLAFAXINE	PRISTIQ
	VILAZODONE	VIIBRYD
	FLUVOXAMINE	LUVOX
PHENOTHIAZINE/ RELATED ANTIPSYCHOTICS	CHLORPROMAZINE	THORAZINE
	FLUPHENAZINE	PROLIXIN, PROLIXIN DECANOATE
	PERPHENAZINE	TRILAFON
	THIOTHIXENE	NAVANE

	THIORIDAZINE	MELLARIL
	MESORIDAZINE	SERENTIL
	TRIFLUOPERAZINE	STELAZINE
ANTIPSYCHOTICS, OTHER	ARIPIPRAZOLE	ABILIFY
	CLOZAPINE	CLOZARIL
	HALOPERIDOL	HALDOL, GALDOL DECANOATE
	LOXAPINE	LOXITANE, LOXAPINE, ADASUVE
	LURASIDONE	LATUDA
	OLANZAPINE	ZYPREXA, ZYPREXA ZYDIS,
	PALIPERIDONE	INVEGA
	QUETIAPINE FUMARATE	SEROQUEL
	RISPERIDONE	RISPERDAL , RISPERIDOL CONSTA
	ILOPERIDONE	FANAPT
	ASENAPINE	SAPHRIS
	ZIPRASIDONE	GEODONE
LITHIUM SALTS	LITHIUM	LITHIUM
AMPHETAMINES	DEXTROAMPHETAMINE	DEXEDRINE, ADDERALL
	AMPHETAMINE	ADDERALL
AMPHETAMINE LIKE STIMULANTS	METHYLPHENIDATE	CONCERTA, RITALIN
	DEXMETHYLPHENIDATE	FOCALIN, ATTENDAE
CNS STIMULANTS, OTHER	CAFFEINE	CAFFEINE, CAFCIT
	DONEPEZIL	ARICEPT
	ERGOLOID MESYLATES	HYDERGINE
	GALANTAMINE	RAZADYNE
	MEMANTINE HCL	NAMENDA
	PIMOZIDE	ORAP
	PEMOLINE	CYLERT
	RILUZOLE	RILUTEK
BETA BLOCKERS/ RELATED	PROPRANOLOL HCL	PROPRANOLOL
ALPHA BLOCKERS/ RELATED	PRAZOSIN	MINIPRESS
ALPH-2 AGONISTS	CLONIDINE	CATAPRES, KAPVAY, DURACLON, NEXICLON
	GUANFACINE	INTUNIV, TENEX

Assessment Detail

Schedule and List of Data Collection Instruments

Instruments and Assessments	Baseline	Mid treat-ment	End treat-ment	1-Mo. Follow up	6-Mo. Follow up
1. Screening, Diagnostics, and Trauma Exposure					
Screening Questionnaires	X				
Clinician Administered PTSD Scale-5 (Blake et al, 1995)	X				
Structured Clinical Interview for Diagnosis (First et al, 2001)	X				
Dissociative Experiences Scale (Bernstein & Putnam, 1986)	X				
Beck Depression Inventory – II (Beck et al, 1996)	X				
Deployment Risk and Resilience Inventory-2 (Vogt et al, 2008)	X				
2. Primary Outcome (Clinical)					
PTSD Severity Score on CAPS-5 (Blake et al, 1995)	X	X	X	X	X
3. Secondary Outcome (Biological)					
PPR (startle)	X		X		X
4. Exploratory Outcomes					
PTSD Checklist-Military (PCL-5) (Weathers et al, 1993)	X	X	X	X	X
Beck Depression Inventory – II (Beck et al, 1996)	X	X	X	X	X
Hopkins Symptom Checklist-25 Anxiety Scale (Derogatis et al, 1974)	X	X	X	X	X
New Mexico Symptom Checklist Somatic Sc. (Hollifield et al, 2009)	X	X	X	X	X
Pittsburg Sleep Quality Index (Buysse et al, 1989)	X	X	X	X	X
Veterans RAND 12-item Health Survey (Selim et al, 2009)	X	X	X	X	X
PPR (HR, HRV, SCR)	X		X		X
5. Safety and Control Data (to be administered weekly or monthly)					
Aggression Questionnaire (Buss et al, 1992)	weekly		X	X	X
Beck Depression Inventory – II items 9, 11, 17	weekly				
Satisfaction with Care and Provider Scale (Unpublished)			X		X
Intercurrent Health Resource Use Inventory	X		X		X
6. Treatment Fidelity and Expectancy					
Treatment Fidelity Assessment (Therapist Adherence Scale)	selected				
The Credibility Rating Scale (Borkovec, 1972)	selected				

Description of Instruments

1. Screening and Diagnostics

The Clinician Administered PTSD Scale (CAPS) (Blake et al, 1995) is a structured diagnostic interview for PTSD. The new CAPS-5 based on DSM-5 criteria will be used (CAPS-5 update, unpublished: Weathers et al, 2013). As with previous versions of the CAPS, CAPS-5 symptom severity ratings are based on symptom frequency and intensity, except for items 8 (amnesia) and 12 (diminished interest), which are based on amount and intensity. However, CAPS-5 items are rated with a single severity score, in contrast to previous versions of the CAPS which required separate frequency and intensity scores for each item that were either summed to create a symptom severity score or combined in various scoring rules to create a dichotomous (present/absent)

symptom score. CAPS-5 has 20 symptom items, each rated from 0 (absent) to 4 (severe). A rating of >2 is considered a positive score for diagnostic purposes. There are 4 symptom clusters: the Criterion B (re-experiencing) severity score is the sum of the individual severity scores for items 1-5; the Criterion C (avoidance) severity score is the sum of items 6 and 7; the Criterion D (negative alterations in cognitions and mood) severity score is the sum of items 8-14; and the Criterion E (hyperarousal) severity score is the sum of items 15-20. The DSM-5 diagnostic rule requires the presence of least one Criterion B symptom, one Criterion C symptom, two Criterion D symptoms, and two Criterion E symptoms in addition to other impairment criteria (see "scoring" on CAPS). A DSM-5 CAPS cutoff score of ≥26 AND meeting each of the 4-symptom cluster criterion will be used for study inclusion. This is consistent with what will be used in the VA Cooperative Study #591, which is the first national study to use the CAPS-5 for diagnosis and treatment effect assessment.

The Structured Clinical Interview for Diagnosis (SCID) (First et al, 2001) is the most frequently used diagnostic interview in psychiatric research for assessing diagnoses. The modules appropriate to inclusion and exclusion criteria will be used in this study by the clinical assessor to verify the presence or absence of PTSD diagnosis and to determine if exclusionary diagnoses are present. The updated DSM-5 version will be used; it is expected to be available by the time of funding/study start, as it is now being used in the national CSP 591 study comparing efficacy of Prolonged Exposure with Cognitive Processing Therapy (The PI for the proposed study is on the executive committee and is a site investigator for CSP 591)

The Dissociative Experiences Scale (DES-II) (Bernstein and Putnam, 1986) is a 28-item self-report scale used to measure the frequency of dissociative experiences using a 10-point Likert scale with verbal quantifiers. Item scores range from 0 to 100. Total scores are calculated by averaging the scores of the 28 items, which refer to amnesia, depersonalization, derealization, absorption, and identity alteration. A score of 30 or more is considered suggestive of severe or pathological dissociation, predicting a diagnosis of Dissociative Identity Disorder (DID) with a sensitivity of .74 and specificity of .80, where a cut-off score of 25 predicted the diagnosis of DID with sensitivity of .93 and specificity .86 (Carlson & Putnam, 1993). A meta-analysis demonstrated the DES-II to have a high test–retest reliability of .78 - .93, an internal reliability (alpha) of .93, and a convergent validity of .67 (Thomson & Jacque, 2011). A score of ≥25 will exclude a potential participant from the study.

The Beck Depression Inventory–II (BDI-II) (Beck et al, 1996) is a self-administered scale that assesses symptoms and severity of depression by summing items (Range 0 – 63; mild = 14 - 19; moderate = 20 – 28; severe = 29 - 63) a study comparing outpatients diagnosed with mild, moderate, and severe depression, respectively, the mean BDI-II total scores were 18 (SD = 8, 99% CI 12-23), 27 (SD = 10, 99% CI 24-29), and 34 (SD = 10, 99% CI 30-37) ($F2.257 = 33.25$, $p<.001$), respectively, demonstrating good validity and no overlap between severity categories (Steer et al, 2001). For this study, BDI-II inclusion criteria score will include the range from zero to the upper end of the 99% CI found valid for predicting moderate depression (i.e., 29). Patients over a score of 30 might be entered if PTSD is clearly primary to depression temporally and clinically in the opinion of the PI and assessor.

Combat Trauma Exposure and Pre-Deployment Preparedness

The Deployment Risk and Resilience Inventory-2 (DRRI-2) (Vogt et al, 2008) is a suite of 17 distinct scales that assesses deployment-related risk and resilience factors. The DRRI-2 is the product of a four-year Department of Veteran Affairs-sponsored research program funded by two consecutive grants from VA Health Services Research and Development Service. The 17 DRRI-2 scales fall into three general categories: predeployment factors, deployment factors, and postdeployment factors. These scales assess independent factors. For this study we will assess 2 of the scales to assist with adaptive randomization: combat experiences (17 items) and preparedness (10 items). Combat experiences assess exposure to objective combat-related circumstances on a 6-point Likert response format (1 = Never; 6 = Daily or almost daily), with a possible range of 17 to 102; higher scores indicative of more combat experiences. Preparedness assesses the extent to which an individual perceives that he/she was prepared for deployment on a 5-point Likert response format (1 = Strongly disagree; 5 = Strongly agree), with a possible range of 10 to 50; higher scores indicative of a stronger sense of

preparedness.

2. Primary Clinical Outcome

PTSD Severity Score. Full-scale PTSD scores from the CAPS-5 will be utilized. The scoring is described above. CAPS-5 also yields continuous scores for the four symptom clusters, which will also be used for analysis of clinical response and association with biomarker change. These clusters are:

Criterion B (re-experiencing): the sum of the individual severity scores for items 1-5

Criterion C (avoidance): the sum of items 6 and 7

Criterion D (negative alterations in cognitions and mood): the sum of items 8-14

Criterion E (hyperarousal): the sum of items 15-20

3. Secondary Outcome (**Startle response** assessed by EMG eyeblink during fear conditioning procedure) and **4.2 Exploratory PPR outcomes** (**PPR of heart rate, heart rate variability, and skin conductance** responses assessed at baseline and fear conditioning procedures). All data will be collected, amplified and digitized by the BioSemi recording software (BioSemi B.V, Amsterdam, Netherlands). Data will be exported to Mindware software (Mindware Technologies LTD, Gahanna, OH) for data reduction and generation of analyzable variables.

Startle. Startle will be measured by administering a 250 ms airblast with an intensity of 140 p.s.i. directed to the larynx as described in similar human fear conditioning studies (Norrholm et al, 2011), recording the participants' electromyographic (EMG) activity during eyeblink muscle contractions. EMG startle eyeblink responses will be recorded using two 5mm Ag/AgCl electrodes placed over the orbicularis oculi muscle of the left eye. One electrode will be placed directly below the pupil in forward gaze while the other will be placed about 1 cm lateral to the first. Both electrodes will be placed as close to the eye as possible while still allowing the participant to close his or her eyes comfortably. Impedance between the two electrodes will be measured and deemed acceptable if below 10 kΩ. Startle responses will be rectified and integrated for analysis using a 20 ms time constant. In order to be scored, the onset of the blink response must occur within a window of 20 to 100 ms after startle probe onset. The blink response must reach peak amplitude within a window of 20 to 150 ms following the startle probe. Amplitudes will be recorded as the difference between the peak activity value and the baseline level that was present immediately preceding onset of the blink response. Participants who fail to reach 1μV amplitudes on more than 50% of probed trials will be considered non-responders and will be excluded from further EMG analyses.

Heart rate. Electrocardiogram (ECG) activity will be recorded throughout the experiment by employing a Lead 1 electrode placement. Electrode sites will be cleaned with alcohol prep pads to improve contact.

Skin Conductance. EDA will be recorded by Ag/AgCl electrodes (Unibase gel) attached to the distal phalanx of index and middle fingers to measure skin conductance level (SCL) and skin conductance response (SCR).

General measurement procedure. All PPR measures (secondary and exploratory outcomes) will be obtained in a single visit at each timepoint. There are two procedures at each visit, and these total 49 minutes of recording time. We expect the total visit with set-up, prep, and wrap-up to take approximately 70 minutes. This includes a 15-minute rest period at the beginning to help diminish anticipatory anxiety.

Procedure	Baseline 5 minutes	Fear Conditioning 44 minutes
Assessed Variable(s)	SC, HRV	Startle, HR, SCR

Baseline procedure (5 minutes). Tonic measures of SC and HRV will be assessed during a five-minute baseline period. Participants will be instructed to sit in a relaxed position but to refrain from moving as much as possible.

Power spectral density analyses of HRV will be performed with use of a fast Fourier transform based algorithm in Matlab. The algorithm will be used to calculate the spectral power of the low frequency (LF) component and the high frequency (HF) component of the HRV measure. The frequency range of the LF component is between 0.04 and 0.15 Hz and is associated with sympathetic activation, while the HF component is between 0.15 and 0.4 Hz and is associated with parasympathetic activation (Task Force of the European Society of Cardiology the North American Society of Pacing Electrophysiology, 1996). We will analyze the LF/HF ratio as a measure of cardiac sympathovagal balance.

Mean and median skin conductance levels will be analyzed during the baseline procedure at pre-treatment, post-treatment, and follow-up timepoints. Additionally, the frequency of non-specific skin conductance responses (NS-SCRs) will be calculated as a measure of sympathetic arousal. NS-SCRs are represented by fluctuations in the electrodermal signal of greater than 0.01 microsiemen (μS).

Fear conditioning procedure (44 minutes). The following methods will allow us to assess fear acquisition, within- and between session extinction, and conditioned inhibition (learned safety), as well as participants' awareness of reinforcement contingencies in the experiment. The aversive stimulus (also known as the unconditioned stimulus; US) in these studies will be a 250 ms airblast with an intensity of 140 p.s.i. directed to the larynx as described in similar human fear conditioning studies (Norrholm et al, 2011). Airblasts will be emitted by a compressed air tank connected to polyethylene tubing and controlled by a solenoid switch. Conditioned stimuli (CS's) will be colored shapes presented on a computer monitor. The colored shapes will be counterbalanced across subjects. Stimuli will be presented using SuperLab 4.0 for Windows (Cedrus, Inc.) and synchronized with psychophysiological data acquisition using a DIO card (Measurements Computing, Inc.).

The fear conditioning paradigm will consist of 2 experimental sessions at each testing timepoint. Testing will occur at baseline, during mid-treatment, post-treatment, and at 6-month follow-up. The fear conditioning sessions, described below, include an Acquisition session based on the AX+/BX- design and an Extinction session:

Acquisition (AX+/BX-) Session. Methodology will be similar to those employed in past studies (Jovanovic et al, 2009). The session will begin with a Habituation Phase consisting of six acoustic startle probes presented alone, referred to as noise alone (NA) trials, to reduce initial startle reactivity. This will be followed by a Pre-exposure Phase, during which the participants will view the shapes (A, B, and X) but they will not be paired with the US. The Acquisition Phase will include 3 blocks with 12 trials (4 AX+, 4 BX-, and 4 NA trials) in each block for a total of 36 trials. The Inhibition Testing Phase will consist of a block of 3 NA trials and 3 trials with A and B presented together. In the AX+ trials, two shapes of different color will be presented together with a "+" between them to encourage elemental processing of the shapes. The shapes will be presented for 6 seconds. The startle probe (40 ms burst of white noise at 106 dB) will occur 6 seconds after shape onset; the shape presentation will co-terminate with the US 500 ms later. BX and AB trials will also contain two different colored shapes, and a startle probe presented 6 seconds after shape onset, but there will be no US in these trials. A Re-conditioning block of 12 trials (4 AX+, 4 BX-, and 4 NA) will be presented at the end of the session to increase the fear response prior to extinction. Inter-trial intervals will be randomized between 9 and 22 seconds.

Extinction Session. After a ten-minute rest period following the completion of the Acquisition session or AX+/BX-, the same shapes will once again be presented in semi-random order. The extinction phase will include 4 blocks of 12 trials (4 AX,4 BX, and 4 NA) in each block for a total of 48 trials. During Extinction, the AX trials will no longer be paired with the presentation of the US. The US will not be presented at any time during the Extinction phase. Startle probes will still be delivered for 40 ms during shape presentation, 6 seconds after shape onset, and will co-terminate with each stimulus. Inter-trial intervals will again be randomized between 9 and 22 seconds.

Under consideration: The above protocol is being used for a current DoD study evaluating the effects of virtual

reality for conducting Prolonged Exposure. In addition to the above protocol, the DoD study is using a "virtual combat zone" at the end of the protocol to assess the change with treatment is psychophysiological response to the virtual zone. As more data about the usefulness of this procedure is available, we will consider adding this component to the protocol in the current proposal.

4. Exploratory Outcomes

Clinical

PTSD. The PTSD Checklist-Military (PCL-M) (Weathers et al, 1993) (PCL-5 update, November 2013, National Center for PTSD) is a 20-item measure designed to assess PTSD symptom severity. Respondents are presented with 20 specific symptoms of DSM-5 PTSD and asked to rate 'how much you have been bothered by that problem in the last month' on a five-point Likert scale, ranging from 0 (not at all) to 4 (extremely). The PCL-M has excellent internal consistency in veterans, victims of motor vehicle accidents and sexual assault survivors (αs >0.94) and excellent test–retest reliability in veterans ($r = 0.96$). The PCL-M may be used as a diagnostic (dichotomous) or continuous measure. The PCL-5 does not have published data yet, but preliminary metric analyses by the National Center for PTSD indicate similar properties (CSP #591 Executive Committee communication, P. Schnurr, July 2014).

Depression. The BDI-II (described above) will be scored as a continuous and ordinal (i.e., mild, moderate, severe) variable for analyses.

Anxiety. The Hopkins Symptom Checklist-25 (HSCL-25) (Derogatis et al, 1974) anxiety scale will be self-administered to assess symptoms of anxiety. This scale includes 10 items scored on a 4-point ordinal severity scale. An item-averaged score $\geq$1.75 has been deemed clinically significant in U.S. clinical and community populations. Both continuous and dichotomous variables will be used in analyses.

Somatic Symptoms. The New Mexico Symptom Checklist Somatic Scale (NMSCL-SOM) (Hollifield et al, 2009) was developed in our previous work on PTSD in refugee populations by combining literature review, research team brainstorming and consensus, and in-depth interviews and focus groups to identify physical symptoms that are associated with PTSD, and are persistent and distressing in the past year. The NMSCL-SOM consists of 39 symptom items drawn from the full scale NMSL-121 administered using a 5-point categorical response scale. Hollifield et al. (2009) reported an alpha coefficient of 0.86, and correlations with standard measures of distress (a posttraumatic symptom scale and HSCL-25) ranging from 0.63 to 0.75 for the full scale. The continuous score will be a secondary outcome in analyses.

Sleep and Dreaming. The Pittsburg Sleep Quality Index (PSQI) assesses sleep quality during the past month based on 7 continuous component scores for sleep quality, latency, duration, efficiency, disturbance, medication use and daytime dysfunction that sum to a global score (range 0-21) (Buysse et al, 1989). The PSQI has been widely used in sleep and PTSD research.

Impairment. The Veterans RAND 12-item Health Survey (VR-12), has 12 items that yield physical (PCS) and mental (MCS) component summary scores that are normed to the U.S. general population (mean of 50 and SD of 10). VR-12 PCS and MCS scores in the Medicare Health Outcome Survey averaged 40 and 50), respectively (Selim et al, 2009). More than 2 million VR-12 questionnaires have been administered in the VA for quality monitoring purposes. Research studies that have used the VR-12 include randomized clinical trials in the VA cooperative studies program, and the nationwide VA Health Study.

Biological (see description above in secondary outcomes)

5. Safety Assessment and Other Control Data

Anger, aggression, and suicidality will be assessed once per week at one of the acupuncture sessions. If a subject is identified as having high scores on any of the items, he or she will be further evaluated by the study PI (MH) for safety and an action plan will be determined.

Anger and Aggression. The Aggression Questionnaire (AQ) (Buss et al, 1992) has 29 items and 4 scales measuring physical aggression, verbal aggression, anger, and hostility. A total score and an inconsistent responding index (lie scale) are also derived. Cronbach's alpha for the total scale was found to be 0.85 and alphas for the scales ranged from 0.72 to 0.85. Test-retest correlations ranged from 0.72 to 0.80. The AQ will be fully administered at the primary data collection time points. To evaluate safety, ten items (1, 2, 5, 8, 11, 13, 18, 23, 25, 28) will be administered weekly as described above.

Suicidality. The Beck Depression Inventory–II (BDI-II) (Beck et al, 1996) items 9 (suicidality), 11 (agitation), and 17 (irritability), will be administered once per week as described. A score of 2 or 3 on any item will prompt further evaluation. Item 9 has been shown to be moderately correlated to the full scale Beck Scale for Suicide Ideation (Beck et al, 1988).

Satisfaction with Care. The Opinions about Care and Provider Scale is adapted from a scale developed by our research team for an NIH-sponsored study. Ten items assess opinions about care and represent satisfaction with care. Five items are designed to assess if the participant was taught specific skills.

Intercurrent Health Resource Use Inventory. This will be administered at baseline, end-of-treatment, and 6-month post treatment to help account for other non-study treatment effects.

6. Treatment Fidelity and Expectancy as Potential Moderators to Outcome

Treatment Fidelity. A Therapist Adherence Scale will be developed in the first 3 study months. It will be like one in the Appendix which was developed for computer assisted CBT

Treatment Expectancy will be assessed with The Credibility Rating Scale (Borkovec, 1972), which has been utilized in many clinical trials including other studies comparing interventions to placebo's in general.

Treatment Fidelity and Subject Expectancy. Assuring treatment fidelity (clinician) is important in clinical trials. This may be accomplished by various methods, dependent on trial design, and often involves standard training, random checks by live or taped observation, and ongoing training to promote reliability. With one practitioner, accuracy and consistency is required, but of course there is no need for inter-therapist reliability. First, the study acupuncturist hired will be well-trained, research experienced, and further trained on study protocol by Dr's. Hollifield and Hsiao. At least five volunteer patients using each ACU and MIN will be treated and observed; landmarks, needle insertion and manipulation, electrical stimulation, and needle removal will be judged and discussed for each case. Second, non-specific elements of the intervention, such as instructions for treatment preparation, and interactions during treatment will be reliably delivered by using clinical scripts adapted from our first study. Third, all treatment sessions will be videotaped. The first 5 and a random selection of 15% (18 total cases) thereafter chosen by a computer randomizer will be scored by study assessor supervised by Dr. Reist using the Fidelity Assessment Rating Form adapted for this study. Dr's. Hollifield and Hsiao will provide feedback to the acupuncturist about improvement. We will use scores to explore score effects by group.

It is important to assess effects of treatment expectancy on outcomes. We do not want to encourage subject learning about type of intervention, so will not assess expectancy after each session. Rather, we will assess treatment credibility (Borkovec, 1972) as has been done in previous acupuncture studies before session 1 and after sessions 2 and 24, and explore effects of expectancy by group and total sample on outcome.

Demographic and Other Data. A demographic and other data form will be developed for this study to assess important information for matching and data analyses: age, gender, ethnicity, combat history, medical history, medications, potential head injury, exposures to toxic chemicals, and current treatment.

Human Subjects Detail

Human Subjects Involvement and Characteristics: This study will utilize a longitudinal randomized design to evaluate the efficacy of 12-week manualized verum acupuncture (ACU) for PTSD in combat Veterans. The primary outcome will be the pre-to post-treatment change in PTSD symptom severity. The secondary outcome will be a peripheral physiological response (PPR) of startle response to a stimulus. Exploratory outcomes will be other PPR indices (biological) and clinical conditions that are associated with PTSD. Potential subjects will be recruited from the Long Beach Veterans Affairs Healthcare System (LBVA) and associated Veteran organizations. We will randomize 90 subjects into the two study groups of 45 each: subjects will be randomized to either: (1) verum acupuncture (ACU), or (2) placebo minimal needling (MIN).

Potential risks: The general risks are primarily psychological. Being asked to recall adverse life events or distressing symptoms in the diagnostic interview may compound the distressing nature of PTSD. However, interviews with survivors of torture and related trauma have found that testimony about traumatic experiences can be therapeutic (Cienfuegos and Monelli 1983; Agger and Jensen 1990; Thompson and McGorry 1995). There is evidence that trauma disclosure is helpful physically and psychologically over time, although in the short-term symptoms of depression may increase (Pennebaker and Susman 1988). The likelihood of the study worsening PTSD is small, since by the nature of the disorder, those who have PTSD will already be "experiencing" their trauma in a variety of ways, and all subjects will be provided with one EBT, the medication paroxetine. Study personnel need to be attentive to population and individual responses.

The physical risks include those from acupuncture, which includes minor bruising, hematoma and bleeding, fainting or nausea. There is a less than 1 in 10,000 risk of pheumothorax, and the reports of this are when the procedure was conducted by inexperienced clinicians. There is no reported risk from the psychophysiological assessment, although the stimulus (an air blast to the neck) is meant to be aversive, and participants have in other studies not wanted to return for testing.

Research risk: PTSD is a chronic illness that creates vulnerability and can lead to potential exploitation of subjects. The study includes interviews and questionnaires about PTSD symptoms and potentially traumatic combat experiences, as well as about other psychiatric symptoms and life events. In many studies involving the collection of information for gaining knowledge (e.g., biological markers), participants may not receive intervention for the condition under study. In this study, 45 subjects with PTSD will receive ACU, an intervention supported by current VA/DOD treatment guidelines for PTSD. Forty-seven will receive MIN, a placebo intervention. There is risk that their PTSD will go untreated, though study protocol will allow evidence-based medication if they are on them at a stable dose for $\geq$8 weeks. There is the risk that any participant will not improve but will either continue to have similar or higher levels of distress and symptoms.

Audio-Video Taping: Subjects will be asked to sign an informed consent to be taped. This consent will detail the use of the tapes (that they will be reviewed for provider treatment review), and that the tapes will be erased after the scoring for treatment fidelity is completed

There is a risk of non-effectiveness. This is more likely to be true for the MIN subjects.

Sources of Materials

Training materials. Any training materials will be available for study staff to use during the project. These materials are products of the project and will be kept in locked offices at the LBVA research offices throughout and after the end of the project.

Participant rosters. The electronic and hard copy rosters with full identifiers (participant names, addresses, and phone numbers) that can be linked to study and treatment ID numbers will be kept in locked file cabinets in research offices that are locked after hours at the LBVA. The electronic files will be password protected. At the end of the project only two copies of the research rosters will be kept in separate locked cabinets at the LBVA

research offices. Original rosters that connect ID numbers with participant identifiers will be destroyed at the end of the project. Electronic and hard copy rosters after the end of the project will be thus de-identified.

Data. Scores and data from psychological and physiological testing will be recorded and utilized solely for research purposes. All data will be entered into an electronic database at LBVA as the project progresses, and similar backup procedures will be employed. All hard copies of data will be marked only with a Study Identification number (SID#) and will be kept in a locked file cabinet in a space that is separate from participant rosters. After the hard coy questionnaire data is entered into the electronic database all original hard copies will be kept in locked research offices at LBVA. Final biological data that is analyzed or reviewed by any of our consultants at other institutions will only be identifiable by SID # and a date of collection number. These data will be sent between institutions by a passcode protected and encrypted email. Data are backed up each day on the main server at the LBVA.

Data Management Plan. The study data analyst (DA) will develop spreadsheets for each assessment in EXCEL under the supervision of the PI and the statistical consultants. The physiological data will be formatted for entry into ASCI files, which can be transferred to either EXCEL or MATLAB sources. The DA will enter and double check all data into respective spreadsheets as the data are collected. Once data entry is completed and merged into the primary database, transfer into SPSS or SAS files will be conducted and initial data runs will be performed to investigate data integrity. Once data integrity is determined, an original set of data files will be stored as "original" in case other iterations are harmed, altered, or lost. All electronic data files will be passcode protected, and only SID numbers and not other identifiers will be on each spreadsheet.

Compensation Plan. Our research team has consulted with participants and professional advisors to determine compensation for participants in this project. The amounts allocated ($25 for initial assessment, $150 for all biological assessments, $25 for follow-up assessment, and $50 for participating in the clinical trial) were thought to be fair given the level of participation and the potential benefit of intervention, and are intended to not be exploitative either from being insufficient for the time and effort spent, or as being coercive by being too large a sum.

2. Adequacy of Protection from Risk

Recruitment and Informed Consent

The methods of recruitment, informed consent, interviewing, and other assessment is intended to be protective for the participants, as they are potentially vulnerable to exploitation and harm given their medical/psychiatric status. We are attentive to ethical protections by: 1) following guidelines for ethically sound research, and 2) by having the capacity at LBVA to review research forms and treatment protocols from both a scientific and ethical perspective. Finally, we will encourage participants to ask questions and include family and friends in the informed consent decision process.

The process of obtaining informed consent is conducted at the Program for Traumatic Stress or a designated research office located at LBVA. Only authorized and properly trained study personnel by the PI are permitted to participate in the actual consent process. Potentials subjects will be asked by research staff if he is interested in participating in the study. If he is interested, research staff will conduct education and ascertain if he wishes to participate. If the potential subject agrees to participate, the subject will be asked to provide informed consent.

Potential subjects will be asked if s/he can understand the consent form by reading the first paragraph. Qualified study personnel will then fully explain each section of the consent form in detail and will ask the potential subject to follow-along with another copy of the consent form. All potential subjects will be properly informed about the purpose of the study, study procedures, and the potential risks and benefits of being a participant. Each potential subject will affirm his or her understanding of each section prior to moving on to the next, and then will be given a copy of the consent form to also read at his leisure. The qualified research

personnel will then ask the subject a number of open-ended questions about his level of understanding to ensure he has processed the information.

Once all questions have been asked and answered to each party's satisfaction, the consent form will be signed by the potential research subject and the qualified study personnel. If a potential subject wishes to think about participation, he will be encouraged to take the consent home, think about it, and discuss it with anyone whom he has a close personal relationship with to assist in making a decision. It will always be stressed that participation is fully voluntary and does not compromise care provided by the LBVA and the Program for Traumatic Stress. If the potential subject agrees to participate in the study, the PM will obtain written informed consent; the subject will be given a signed and dated copy of the consent form for his record. Once informed consent has been obtained, a screening for inclusion and exclusion criteria will be conducted to confirm eligibility for the study. The PM, who obtained informed consent, will document into the subject's electronic file in Computerized Patient Record System (CPRS) that informed consent was obtained.

Protections Against Risk

All research team members, including investigators, consultants, contractors and staff will be asked to sign a statement that details their conduct regarding participant confidentiality. This statement details consequences to team members for breach of confidentiality.

Our research staff is or will be trained to be open to participant questions and concerns. The participants will be encouraged to have an ongoing discussion and dialogue with the clinicians and staff. It will be made clear to each participant in the informed consent process and beyond that participation is voluntary, and that he may withdraw at any time without adverse consequences to his usual health care.

We recognize the variability of individual responses to answering questionnaires and interview questions related to PTSD, other life events, symptoms, and current health and social status. Team members will be sensitive and helpful to any individual participant who is distressed, whether or not the distress is from a research effect. The PM at LBVA is very experienced with and will field all calls and inquiries from participants about urgent treatment needs. If it is PTSD or study related, the PM will immediately troubleshoot the potential problem with the PI. If it is emergent, the PM will help the participant make a plan for a visit, which may include calling emergency transportation.

Adverse effects are extremely rare when acupuncture is performed in a professional setting by a fully trained and certified acupuncturist. Four out of the 5 deaths that have been associated with acupuncture in the literature were not equivocally due to acupuncture or were either self administered or administered by an untrained person. Possibilities of cross-infection will be avoided by using disposable needles and following clean-needle technique procedures. Other adverse effects will be avoided by using a fully trained and licensed acupuncturist who will practice in a professional setting.

The risk of non-effectiveness is mitigated by subjects being allowed to be taking medications for symptoms of PTSD and related conditions. Participants will be seen twice per week for protocol, and will be assessed each visit for aggression and suicidal ideation. Rules for withdrawal from study because of symptoms and/or potential danger to self or others will be part of protocol.

The video tapes will be destroyed after they have been reviewed for treatment fidelity.

Any adverse events will be documented and reported to proper authority. All research staff has been or will be trained in and abide by ethical research methods and good clinical practices.

3. Potential Benefits

There may be no direct benefit for a person to participate in this study, although we hypothesize benefit to subjects in the ACU group. The risks are minimal but present. For this study, an emphasis on informed consent

and safety is important. Results from this study are intended to provide a better understanding of the effects of acupuncture on PTSD and on peripheral psychophysiology. This information may enhance knowledge of and shape the treatment for PTSD which may potentially help thousands of veterans suffering from this illness.

4. Importance of Knowledge to be Gained

This study will improve knowledge about the use of acupuncture for PTSD, and will inform the field and policy makers about how acupuncture may be useful treatment for symptoms and biological functioning. Research evaluating how abnormal biological function in PTSD might be reversible with intervention is rare, and this study will help us understand what is reversible and what is not. This may improve our knowledge about mechanisms of treatment, and perhaps provide insight into risk factors. This will hopefully provide potential biological targets for further study.

5. Data and Safety Monitoring

Subjects will be given a sequential ID number that does not identify their PTSD status or their group assignment. Electronic and hard copy rosters with full identifiers (participant names, addresses, and phone numbers) that can be linked to study numbers will be kept in locked file cabinets in research offices that are locked after hours at LBVA. The electronic files will be password protected. At the end of the project, original rosters that connect ID numbers with participant identifiers will be destroyed.

In past studies in other settings we have constructed an internal Data and Safety Monitoring Committee or Board depending on the trial. We recognize and are pleased to know that, with funding via a Merit Review from CSR&D, we are eligible to use the services of the Centralized Data Monitoring Committee (DMC), and we are pleased that this study will have the availability of oversight by a CSR&D Centralized DMC panel.

The study protocol and requests for research review are pending submission to the Institutional Review Board at the VA Long Beach Healthcare System. The IRB at the VA Long Beach meets twice a month, and so the protocol will be able to be reviewed and appropriately approved within the first three months after funding.

To ensure adequate subject recruitment and enrollment, we will utilize recruitment flyers and approach subjects and clinicians at the Program for Traumatic Stress, in other mental health clinics, at Vet Centers and CBOC's to introduce the project and ask clinicians to refer any patients that may be interested in the study. We have developed this system of recruitment in the context of a current clinical trial.

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