Closed Loop Vagal Stimulation in Patients with Posttraumatic Stress Disorder

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PROTOCOL – Phases 1, 2, and Longitudinal Study

Title of Project: Closed Loop Vagal Stimulation in Patients with Posttraumatic Stress Disorder
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Introduction:

This project, funded by DARPA BAA-15-35 (phases 1 and 2), Brain & Behavior Research Foundation, and electroCore, LLC (Longitudinal Study), aims to develop the fundamental physiological understanding required for ultimately improving the quality of life for patients with Posttraumatic Stress Disorder (PTSD) through feedback controlled vagal nerve stimulation (VNS). The current protocol covers phase 1 which involves assessment of healthy individuals with a history of psychological trauma over 12 months and Phase 2 which applies to the study of PTSD patients in a second twelve month period and will be performed only if approved by the sponsor.

The tasks of the project are to map the potency and kinetics of the neurologic, autonomic peripheral, inflammatory, and behavioral responses to VNS (vs. sham treatment), at baseline and in response to stressful traumatic scripts related to personal traumatic events, as well as a series of other stressors.

Our project is structured as a collaborative effort between psychiatry, radiology, epidemiology, and cardiology researchers at Emory University, as well as researchers from the School of Electrical and Computer Engineering (ECE) at the Georgia Institute of Technology (GT). Two of the physicians also have clinical appointments at the Atlanta Veterans Affairs Medical Center. Importantly, our team has access to a high-resolution positron emission tomography (HR-PET) scanner, of which there are fewer than 10 in the world. We call this brain-research dedicated camera "highest resolution" because the 2 mm spatial resolution is the highest possibly obtainable given the minimum distance travelled of positrons emitted from radiolabeled water used in the measurement of brain blood flow. We will leverage this scanner's capabilities to image the brain during VNS, and, particularly, in response to the acute mental stress challenges. In addition to obtaining superior spatial resolution, HR-PET does not require magnetic pulses (as with functional MRI (fMRI)) for measurement of brain blood flow, obviating the technical challenges involved in obtaining simultaneous physiological recordings and delivering electrical stimulation with VNS devices during scanning sessions. Subjects with metallic implants and claustrophobia are also better candidates for HR-PET. HR-PET measurement of brain function thus facilitates the mechanistic understanding of the effects of VNS in the context of mental stress resilience better than fMRI and in a wider panel of subjects.

We have assembled an outstanding team of researchers from these two world class institutions located in Atlanta, GA, with complementary expertise that can lead this project to success: Professor J. Doug Bremner (Emory, Psychiatry and Radiology) is the PI; Professor Amit Shah (Emory, Cardiology and Epidemiology) and Professor Omer Inan (GT, ECE) are the Co-PIs. Additional expertise in Professor Viola Vaccarino, an internationally recognized expert in PTSD and cardiovascular epidemiology, and Associate Professor Brad Pearce, an expert on immunology and blood-based biomarkers of stress, will also further bolster this study as co-investigators from Emory. Additionally, our team has acquired the support of an industry partner, electroCore, LLC and will be working with their team to maximize the understanding of mechanisms of VNS in this effort for improving PTSD patient care. ElectroCore manufactures the gammaCore-R device which delivers non-invasive VNS stimulation for preclinical investigations, clinical studies, or therapeutic use worldwide. The device is approved for use in the European Union and has been granted a CE mark there for bronchoconstriction, cluster, and migraine headache (acute and prophylactic treatments), anxiety, and depression, epilepsy and functional gastrointestinal disorders including IBS and gastroparesis. Preliminary studies show

evidence of similar efficacy to invasive VNS without the risks of surgery. ElectroCore will ensure that the necessary active and sham devices, and supporting material for the products, are available for the duration of these studies.

Posttraumatic Stress Disorder

Novel therapies for PTSD could greatly improve the quality of life and care for millions of Americans, including veterans [1]. In the general population PTSD has a lifetime prevalence of 10-12% in women and 5-6% in men [2]. In veterans, the prevalence of PTSD is at least double that of the general population. PTSD is characterized by hyperarousal, avoidance, anxiety, intrusions, and depression. Furthermore, only *one third* of those suffering from PTSD are able to achieve full remission with the current standard of care, which includes exposure therapy and antidepressants, for example. Unfortunately, these therapies largely ignore the psychobiology of PTSD, involving core changes in brain anatomy and autonomic nervous system (ANS) function [3]. Targeted therapies focused on pathological neurophysiologic pathways may ultimately lead to a significantly higher remission rate (>75%) because they are focused on the core essentials of the PTSD disease state.

Vagal Nerve Stimulation (VNS)

VNS is a potentially highly promising neuromodulator therapy for PTSD that may directly impact core pathophysiology. Currently, implantable VNS therapy is FDA-approved to treat medically refractory epilepsy and treatment resistant depression [4,5], but the same paradigm may be used in PTSD to counteract its characteristic hyperarousal episodes [6]. VNS is efficacious for chronic depression, which is often seen together with PTSD [4], and preliminary open label results showed promise for patients with anxiety disorders including at least one subject with PTSD [7]. Recent studies suggest that VNS therapy for epilepsy is also associated with reduced sympathetic/parasympathetic ratio [8] and arrhythmia risk, although larger, more definitive studies that assess the autonomic effects in a rigorous manner are required. The disadvantages of the currently approved implantable VNS therapy are cost ($30,000), the fact that most insurance policies will not cover it, and the risk and discomfort associated with surgery. This has limited utilization of VNS for the treatment of depression and anxiety. The hand-held gammaCore is safe, effective, convenient, and much more economical than the implanted device.

VNS using the gammaCore involves electrical stimulation with a hand-held device directly to the neck just medial to the sternocleidomastoid muscle, where the vagus nerve travels along through the carotid sheath on its way to the brain.

The vagus nerve is mostly afferent, relaying sensory activity of the visceral organs to the brain through the nucleus tractus solitarius in the medulla oblongata. Efferent branches of the vagus, however, also have important effects, including modulation of inflammatory function [9] through anti-inflammatory effects [10]. The neuro-inhibitory and anti-inflammatory effects of the vagus may have tremendous benefit in PTSD [11,12]. Projections of the vagus through the nucleus tractus solitarius (NTS) extend to the locus ceoruleus and hypothalamus, key areas involved in sympathetic hyperarousal in PTSD [13], as well as brain areas like the amygdala that are involved in the fear response [14]. IL1B, IL-6, TNF, IFNg and CRP are elevated in PTSD (according to a recent meta-analysis), and several of these immune mediators are increased after acute stress [15,16]. High mobility group protein B 1 (HMGPB1) is a proinflammatory master mediator, which is increased in PTSD, and inhibited by VNS [17,18]. T helper cell differentiation is partly controlled by cholinergic neurotransmission [19]. Dysregulation of TH cells differentiation and function has been proposed in PTSD [20,21]. TH1 cytokines include proinflammatory mediators (IFNg) as well as IL-2 and IL-3. TH2 cytokines are IL-4, IL-5, and IL-13. IL10 is stimulated by catecholamines, is broadly anti-inflammatory, and induces a shift in the TH1/TH2 balance toward TH2 dominance. The

TH17 subset is generally proinflammatory and secretes IL-17, and IL-22. Activation of the a7 nAChR decreases TH17 responses [19]. Several studies have suggested that PTSD is linked to a dysregulation of the TH1/TH2/TH17 balance. In combat veterans with PTSD, the percentage of Th1 cells and Th17 cells were increased [21]. The close links of these inflammatory mediators with the HPA axis affirms their role in acute stress pathways [15,16,22]. Although both afferent and efferent arms of the vagus may contribute to the reduction in these proinflammatory cytokines, selective unidirectional stimulation of the cervical vagus has been shown to dampen TNF production [23].

Other cytokines are relevant to stress and may by modifiable by VNS therapy. RANTES (CCL5) *is a* chemokine elevated in PTSD and decreased by nicotinic receptor activation [21,24]. MIF is a novel neuroimmune modulator that overrides the action of glucocorticoids on multiple cell types. It is released from the pituitary in acute stress in animal models. It regulates neurogenesis in the hippocampus, which plays an important role in PTSD [25]. MIF could influence glucocorticoid sensitivity in PTSD. MIF is inhibited by the efferent cholinergic anti-inflammatory pathway [26].

The kynurenine pathway is also relevant to the effects of VNS on physiological function. Activation of the IDO pathway increases Kynurenine (KYN), which crosses BBB (high KYN is linked to depression and suicide. In VNS kynurenine showed a tend level reduction [27], and anthranilic acid (AA), which is neuroprotective, was significantly increased by VNS in humans [28]. Kynurenic acid (KYNA) is an antagonist of alpha 7 Nicotinic receptors which are key mediators of the efferent cholinergic anti-inflammatory loop [19,23,29].

Other neuro-biomarkers of interest to the effects of VNS on stress are Brain Derived Neurotrophic Factor (BDNF) and S100B. A single-nucleotide polymorphism in the BDNF gene (Val66Met) is associated with PTSD, and plasma levels of BDNF are elevated in veterans with PTSD [30]. Combat-training stress increases S100B along with TNF and IL-6 [31].

The hypothalamic-pituitary-adrenal (HPA) axis plays an important role in stress and PTSD [32-36]. Despite controversy in the literature, there is clear evidence that HPA feedback loops are dysregulated in PTSD [36,37]. A few studies have directly tested glucocorticoid receptors (GR), and numerous lines of evidence indicate that these receptors are hyper-sensitive in PTSD [36,38]. In addition to profiling temporal changes in ACTH and cortisol, we will culture immune cells stimulated with LPS and test for the ability of a glucocorticoid agonist (dexamethasone) to suppress the production of pro-inflammatory cytokines. This provides a measure of in vitro production of proinflammatory molecules with and without an immune stimulus (LPS), as well as a complete dose response curve for dexamethasone to examine GR sensitivity. Vagal afferents are known to carry immunosensory signals and activate the HPA axis [39,40]. Rodent studies suggest that VNS may facilitate HPA axis hemostasis [41].

Catecholamine function is critical to the stress response. VNS regulates catecholaminergic function, and dysregulation of this stress-responsive system has been studied extensively in PTSD [42-44]. While many of the details have not been resolved, an abnormal sensitization of the noradrenergic system is generally believed to underlie at least aspects of PTSD pathogenesis [32]. All of the immune, neuropeptide, and neurohormonal systems reviewed above are stress responsive systems that are measured in our comprehensive assessment of biomarker response to stress in the current project.

After the development and validation of invasive VNS, other technologies have been developed that allow for stimulation of the vagus nerve non-invasively. The gammaCore device (electroCore, LLC, Basking Ridge, NJ) has been tested in patients afflicted with intractable cluster headache, episodic migraine, and chronic migraine, with promising results [45-48]. It selectively stimulates low-threshold myelinated vagal afferent A fibers, but not higher-threshold C fibers, of the vagus nerve [49]. Preliminary data show with the gammaCore show similar EEG (evoked potential, or EP) patterns to that seen with implanted VNS. This specific EP pattern has been validated in animal studies as representing a specific

response to stimulation of the vagus nerve [50,51].. The gammaCore device will likely produce similar biological responses to implantable VNS with less safety risks [52]. An option in the current application is to validate the gammaCore device against the gold standard of the implanted VNS device using a physiological marker sensitive to VNS as determined in Phase 1.

The technology we will develop for this project will have broad impact in improving the quality of care for patients with PTSD. The protocol will assess the effects of VNS on brain and biomarker function at baseline and in response to scripts related to subjects personal traumatic events. The fundamental mechanistic advances in the understanding of how VNS impacts trauma recall will, in the future, be leveraged to develop feedback-controlled therapies for PTSD patients. The need for such advances is widely recognized in industry and academia, and is currently a major obstacle in the adoption of VNS in treating PTSD, although its potential has been recognized in the existing literature. Furthermore, our team has a strong background in designing and manufacturing successful commercial products and developing effective intellectual property (IP) portfolios. The findings from this project will likely result in IP that can be licensed by manufacturers of non-invasive and implantable VNS devices.

Brain Regions Implicated in PTSD:

The hippocampus, which plays a critical role in declarative (or explicit) memory (Squire, 2004), is very sensitive to stress. Preclinical and clinical studies have shown that PTSD patients have alterations in memory function (Elzinga & Bremner, 2002). Animals studies corroborate such findings, demostrating that exposure to repeated stress results in deficits to memory function (Luine, Villegas, Martinez, & McEwen, 1994) and damage to the hippocampus (Sapolsky, Uno, Rebert, & Finch, 1990; Uno, Tarara, Else, Suleman, & Sapolsky, 1989). In addition, stress has been shown to interfere with hippocampal-based mechanisms of memory function, including long-term potentiation (LTP) (Diamond, Fleshner, Ingersoll, & Rose, 1996; Luine et al., 1994). Damage to hippocampal neurons may be a result of elevated levels of glucocorticoids (Lawrence & Sapolsky, 1994; Sapolsky, 1996), inhibition of brain-derived neurotrophic factor (BDNF; Nibuya, Morinobu, & Duman, 1995; Smith, Makino, Kvetnansky, & Post, 1995), changes in serotonergic function (McEwen et al., 1992), or inhibition of neurogenesis (growth of new neurons)(Fowler, Liu, Ouimet, & Wang, 2002; Gould, McEwen, Tanapat, Galea, & Fuchs, 1997) in the hippocampus.

However, animals studies have also demonstrated that several agents may reverse or block hippocampal damage due to stress. For example, antidepressant treatments have been shown to block the effects of stress and/or promote neurogenesis in the brain (Czéh et al., 2001; D'Sa & Duman, 2002; Duman, Heninger, & Nestler, 1997; Duman, Malberg, & Nakagawa, 2001; Garcia, 2002; Lucassen, Fuchs, & Czéh, 2004; Malberg, Eisch, Nestler, & Duman, 2000; McEwen & Chattarji, 2004; Nibuya et al., 1995; Santarelli et al., 2003; Watanabe, Gould, Cameron, Daniels, & McEwen, 1992).

Other brain structures often implicated in the neural circuitry of stress and PTSD, include the amygdala and prefrontal cortex (McLaughlin, Baran, & Conrad, 2009). The amygdala is involved in processing of emotional stimuli, emotional memory, and plays a critical role in the acquisition of fear responses (Davis & Whalen, 2001; Davis, 1992; Phelps, 2006). Animal (LeDoux, 2003) and human studies (Phelps, 2006) both demostrate that the amygdala plays a key role fear conditioning. Furthermore, studies of PTSD patients typically show increased activity in the amygdala in response to threat stimuli compared to individuals who did not experience trauma and individuals who experienced trauma but did not develop PTSD (J. Douglas Bremner, 2007a; Protopopescu et al., 2005; Rauch, Shin, & Phelps, 2006).

The final brain region implicated in the neural circuitry of PTSD is the medial prefrontal cortex, which includes the anterior cingulate gyrus (Brodmann's area 32), subcallosal gyrus (Brodmann's area 25), anterior prefrontal cortext (Brodmann's area 9), and the orbitofrontal cortex (Devinsky, Morrell, & Vogt, 1995; Vogt, Finch, & Olson, 1992). The medial prefrontal cortex is implicated in the appraisal and regulation of emotions (Etkin, Egner, & Kalisch, 2011; Quirk & Beer, 2006). Lesion studies demonstrate that the medial prefrontal cortex modulates emotional responsiveness by inhibiting amygdala function (Morgan, Romanski, & LeDoux, 1993). In addition, animal studies show that neurons of the medial prefrontal cortex actively inhibiting fear responses mediate by the amygdala (Milad & Quirk, 2002; Milad, Rauch, Pitman, & Quirk, 2006). Moreover, early stress exposure is associated with a decrease in branching of neurons in the medial prefrontal cortex (Brown, Henning, & Wellman, 2005; Cook & Wellman, 2004; Radley et al., 2004).

Neuroimaging in PTSD

Neuroimaging has been used to study brain correlates of PTSD [53]. Several studies from our group and others found a reduction in MRI-based hippocampus volume in patients with both combat and abuse-related PTSD, which were associated with deficits in hippocampal-based verbal declarative memory [54-59]. Studies in children with PTSD [60] and in new onset PTSD [61] did not find smaller hippocampal volume, suggesting that chronicity of illness or developmental stage may influence the effects of stress on the hippocampus. We also found evidence for a failure of hippocampal activation during declarative memory tasks in PTSD as measured with positron emission tomography (PET).[62]. Functional neuroimaging studies, performed to measure brain function in PTSD, are consistent with dysfunction in a network of related brain regions including the hippocampus, amygdala, and medial prefrontal cortex (J. D. Bremner, 2002; J. Douglas Bremner, 1998; Cannistraro & Rauch, 2003; Liberzon, Britton, & Luan Phan, 2003; Liberzon & Martis, 2006; Liberzon & Phan, 2003; Pitman et al., 2001; Rauch et al., 2006). This network mediates memory and the stress response, and may mediate symptoms of PTSD (J. D. Bremner, 2002, 2003; Pitman et al., 2001). Studies of resting blood flow or metabolism with PET and single-photon emission computed tomography (SPECT) showed alterations at rest in medial prefrontal, temporal, and dorsolateral prefrontal cortex, cerebellum, amygdala (Bonne et al., 2003; J. Douglas Bremner, Innis, et al., 1997; Chung et al., 2006), thalamus (S. J. Kim et al., 2007), and mid-cingulate (Shin et al., 2009).

Stimulating the noradrenergic system with yohimbine, results in decreased activation in cortical regions including dorsolateral prefrontal, temporal, parietal, and orbitofrontal cortex, along with decreased activity in the hippocampus (J. Douglas Bremner, Innis, et al., 1997). PET, SPECT, or fMRI studies, where patients were exposed to traumatic reminders in the form of traumatic slides and/or sounds, or traumatic scripts resulted in an increase in PTSD symptoms, as well as decreased blood flow and/or decreased activation in the medial prefrontal cortex/anterior cingulate, including Brodmann's area 25, or subcallosal gyrus, area 32 and 24 (J. Douglas Bremner, Narayan, et al., 1999; J. Douglas Bremner, Staib, et al., 1999; Britton, Phan, Taylor, Fig, & Liberzon, 2005; Fonzo et al., 2010; Hopper, Frewen, van der Kolk, & Lanius, 2007; Hou et al., 2007; Lanius et al., 2001, 2003; Liberzon et al., 1999; Ramón JL Lindauer, Booij, et al., 2004; Phan, Britton, Taylor, Fig, & Liberzon, 2006; Semple et al., 2000; Shin et al., 1997, 1999, 2001, 2005; Shin, Orr, et al., 2004; Yang, Wu, Hsu, & Ker, 2004). Traumatic reminder exposure has also been shown to decrease function in the hippocampus (J. Douglas Bremner, Narayan, et al., 1999), thalamus (Ruth A. Lanius et al., 2001, 2003), visual association cortex (J. Douglas Bremner, Narayan, et al., 1999; Ruth A. Lanius et al., 2001, 2003; Shin et al., 1997; Shin, Orr, et al., 2004), parietal cortex (J. Douglas Bremner, Narayan, et al., 1999; Rauch et al., 1996; Sakamoto et al., 2005; Shin et al., 1997, 1999), and inferior frontal gyrus (J. Douglas Bremner, Narayan,

et al., 1999; Ruth A. Lanius et al., 2003; Rauch et al., 1996; Sakamoto et al., 2005; Shin et al., 1997, 1999, 2001). An increase in function is observed in the amygdala (Liberzon et al., 1999; Rauch et al., 1996; Shin, Orr, et al., 2004), insula (Fonzo et al., 2010; Hopper et al., 2007; A. N. Simmons et al., 2008), posterior cingulate cortex (J. Douglas Bremner, Narayan, et al., 1999; J. Douglas Bremner, Staib, et al., 1999; Ruth A. Lanius et al., 2001; Shin et al., 1997), ventromedial prefrontal cortex (Morey, Petty, Cooper, LaBar, & McCarthy, 2008), and parahippocampal gyrus (J. Douglas Bremner, Narayan, et al., 1999, 1999; Liberzon et al., 1999).

Studies have used a variety of paradigms to assess amygdala function in PTSD. Admon et al., (2009) showed that increased amygdala reactivity predicted increased stress symptom response to combat. Other neuroimaging studies showed increased amygdala and parahippocampal function while performing an attention task (K. L. Felmingham et al., 2009; Semple et al., 2000), and increased amygdala activity at rest (Chung et al., 2006), during a working memory task (Richard A. Bryant et al., 2005), and whilst recalling traumatic words (Protopopescu et al., 2005). Increased amygdala function is also observed with exposure to masked fearful faces (Armony, Corbo, Clément, & Brunet, 2005; Bryant et al., 2008; K. Felmingham et al., 2010; Kemp et al., 2007; 2009; Rauch et al., 2000), overt fearful faces (Fonzo et al., 2010; Shin et al., 2005), negative pictures (Brohawn, Offringa, Pfaff, Hughes, & Shin, 2010), neutral pictures (Brunetti et al., 2010), traumatic sounds (Liberzon et al., 1999; Pissiota et al., 2002), traumatic scripts (Rauch et al., 1996), extinction learning (Milad et al., 2009), and classical fear conditioning (J. Douglas Bremner, Vermetten, et al., 2005).

In addition to looking at the neural responses to traumatic reminders, several studies have examined the neural correlates of cognitive tasks in PTSD. Decreased activation in the hippocampus (Astur et al., 2006; J. Douglas Bremner, Vythilingam, Vermetten, Southwick, et al., 2003; Shin, Shin, et al., 2004) and insula (Chen, Li, Xu, & Liu, 2009; Whalley, Rugg, Smith, Dolan, & Brewin, 2009) was observed while PTSD patients performed specific declarative memory tasks. PTSD patients showed decreased inferior frontal cortex (Clark et al., 2003), parietal (Richard A. Bryant et al., 2005; Clark et al., 2003), hippocampal and anterior cingulate function (Moores et al., 2008) during working memory tasks. PTSD patients peforming an executive processing task with emotional combate-related scenes which were interleaved in the task showed increased activation in the amygdala, ventrolateral prefrontal cortex, and fusiform gyrus, and decreased activation in the dorsolateral prefrontal cortex (Morey et al., 2008). When performing a same-different emotional conflict task, PTSD patients showed reduced anterior cingulate activation (M. J. Kim et al., 2008). Peri-amygdala areas, ventrolateral prefrontal cortex, and orbitofrontal cortex showed increased activation in patients with high symptomatology during an emotional oddball task (Jasmeet Pannu Hayes, LaBar, Petty, McCarthy, & Morey, 2009). While performing working memory tasks, PTSD patients showed a relatively lack, compared to controls, of connectivity in the right inferior frontal gyrus and right inferior parietal lobule. PTSD patients, however, showed stronger connectivity between posterior cingulate cortex and right superior frontal gyrus, and between medial prefrontal cortex and the left parahippocampal gyrus during the working memory tasks (Daniels et al., 2010). Finally, during an emotional stroop task (e.g., naming the color of a word such as "rape") PTSD patients showed decreased function in medial prefrontal cortex/anterior cingulate (J. Douglas Bremner, Vermetten, Vythilingam, et al., 2004; Shin et al., 2001), visual association cortex and parietal cortex (J. Douglas Bremner, Vermetten, Vythilingam, et al., 2004), and dorsolateral prefrontal cortex (J. Douglas Bremner, Vermetten, Vythilingam, et al., 2004; Shin et al., 2001). Increased function, meanwhile, was observed in posterior cingulate and parahippocampal gyrus (Shin et al., 2001).

Memory studies, where women with PTSD from early abuse retrieved emotionally valenced words (e.g., "rape-mutilate") (J. D. Bremner et al., 2001) resulted in decreases in blood flow in areas

including orbitofrontal cortex, anterior cingulate, and medial prefrontal cortex (Brodmann's areas 25, 32, 9), left hippocampus, and fusiform gyrus/inferior temporal gyrus (J.Douglas Bremner, Vythilingam, Vermetten, Southwick, et al., 2003), thus lending further support to the theory of a disfunctional network of brain regions, including areas implicated in memory, in PTSD. PTSD patients showed decreased frontal, temporal (Elbert Geuze, Vermetten, Ruf, de Kloet, & Westenberg, 2008), and precuneus activation (Elbert Geuze, Vermetten, de Kloet, & Westenberg, 2008) during neutral word encoding. Neutral word retrieval, on the other hand, was associated with decreased activation of hippocampus, middle temporal gyrus, and frontal cortex (Elbert Geuze, Vermetten, Ruf, et al., 2008) and parahippocampal gyrus (Hou et al., 2007). During an associative learning task, where participants had to pair faces with professions, PTSD patients showed increased hippocampal activation and decreased prefrontal activation. During retrieval of the face-profession pairs, patients showed decreased activation in left parahippocampal gyrus and other memory-related brain regions despite showing no differences in memory accuracy, suggesting PTSD may effect memory-related brain function without necessarily impairing memory performance (Werner et al., 2009).

Finally, various neuroimaging studies have looked at the neural correlates of treatment response in PTSD. PTSD patients treated with paroxetine (i.e., Paxil), an SSRI antipressant drug, for up to a year showed significant improvements in verbal declarative memory and a 4.6% increase in mean hippocampal volume (Vermetten, Vythilingam, Southwick, Charney, & Bremner, 2003). Increases in hippocampal volume was also observed following treament with setraline (i.e., Zoloft), another SSRI antidepressant (Letizia Bossini et al., 2007). PSTD patients treated with phenytoin (i.e., Dilantin), a antiepileptic drug that may be efficacious in treating PTSD through antiglutamatergic effects, has been shown to increase right hippocampal and cerebral cortical brain volume (J. Douglas Bremner, Mletzko, et al., 2005). Patients who did not respond to eye movement desensitization and reprocessing (EMDR) therapy—a common therapy that attempts to form associations between traumatic memories and new more adaptive memories and information—showed lower grey matter density compared to patients who responded to EMDR in bilateral posterior cingulate, anterior insula, anterior parahippocampal gyrus, and amygdala in the right hemisphere (Nardo et al., 2009). SPECT studies show that patients with subthreshold PTSD had significant increases in perfusion following psychotherapy as measured with SPECT HMPAO in parietal lobes, left hippocampus, thalamus, and left prefrontal cortex during memory retrieval (Peres et al., 2007). PTSD patients, on the other hand, showed decreased frontal and hippocampal perfusion with SPECT HMPAO, compared to controls who responded to EMDR treatment (Pagani et al., 2007). Treatment of PTSD with EMDR also resulted in decreases in perfusion in the left and right occipital lobe, left parietal lobe, and right precentral frontal lobe, whereas significant increased perfusion in the left inferior frontal gyrus as measured with SPECT HMPAO (K. Felmingham et al., 2007). Successful cognitive behavior therapy (CBT) treatment—which aims to change how patients think about their trauma—resulted in decreased amygdala response and increased anterior cingulate response to fearful faces in PTSD patients (K. Felmingham et al., 2007). Patients with PTSD who responded to CBT had larger rostral anterior cinculate (rAVCC) volumes than non responders (Richard A. Bryant, Felmingham, et al., 2008), whereas those who did not respond well to CBT showed increased amygdala and ventral anterior cingulate cortex activation to masked fearful faces relative to patients who responded well to CBT (R. A. Bryant, Felmingham, et al., 2008). In sum, mounting evidence suggests that successful treatment of PTSD is associated with changes in brain areas that have been implicated with PTSD, including the hippocampus and prefrontal cortex.

Objectives:

- The purpose of this study is to measure neural and biomarker correlates of vagal nerve stimulation VNS in healthy subjects with a history of exposure to psychological trauma (N=40) at baseline and in response to stress. This represents Phase 1 of the study. In phase 2, we will repeat the assessments in a PTSD population (n=40). We hypothesize a decrease in amygdala function with VNS.
- Brain function will be measured with positron emission tomography (PET) and biomarkers will be measured in blood, with an assessment of a broad range of stress responsive sympathetic, hormonal and immune markers.
- Characterize the kinetics of the peripheral physiological responses to VNS through a two day protocol with exposure to a series of naturalistic stressors in conjunction with VNS or sham treatment. The goal of this research is to ultimately determine feedback-controlled optimal timing intervals between administering the therapy to maximize benefit while minimizing negative side effects.
- In the Longitudinal Study, we will follow a subset of the Phase 2 PTSD subjects (n=18).

Patient Selection:

Eligibility Criteria – Phase 1

- Subjects aged 18-70 who, 1) do not meet criteria for PTSD or other major mental disorder as determined by the Structured Clinical Interview for DSM-5 (SCID) interview for PTSD [63] 2) have a history of psychological trauma as defined by DSM-5.

- Eligibility Criteria – Phase 2 and Longitudinal Study
  Subjects aged 18-70 who, 1) meet criteria for PTSD as determined by the Structured Clinical Interview for DSM-5 (SCID) interview for PTSD. 2) For the Longitudinal study, subjects must have a smartphone.

Exclusion Criteria – Phases 1, 2, and Longitudinal Study

- Subjects will be excluded with: 1) positive pregnancy test; 2) meningitis; 3) traumatic brain injury; 4) neurological disorder or organic mental disorder; 5) history of loss of consciousness greater than one minute; 6) alcohol abuse or substance abuse or dependence based on the SCID within the past 12 months; 7) current or lifetime history of schizophrenia, schizoaffective disorder, or bulimia, based on the SCID; 8) a history of serious medical or neurological illness, such as cardiovascular, gastrointestinal, hepatic, renal, neurologic or other systemic illness; 9) evidence of a major medical or neurological illness on physical examination or as a result of laboratory studies (CBC, BUN, creatinine, blood sugar, electrolytes, liver and thyroid function tests, urinalysis, and EKG); 10) active implantable device (i.e. pacemaker); 11) carotid atherosclerosis; 12) cervical vagotomy. Women will be counseled about the risks of pregnancy during the course of the study.

Assessments and Procedures (Phases 1 and 2):

- Structured Interviews and Questionnaires, patients will undergo an initial screening and a structured interview to ensure eligibility.
- Sociodemographic factors. Information on age, race/ethnicity, marital status, education and income will be collected using standard questions from population studies.

- Clinical information including current medications, medical history, etc., will be assessed using standardized questions.
- The PTSD Checklist (civilian version) is a standardized self-report rating scale for PTSD comprising 17 items that correspond to the key symptoms of PTSD.[64,65] This scale is used for screening individuals for PTSD, for diagnosing PTSD, and for monitoring symptom change during and after treatment. There are two versions of the PCL: military and civilian version. The PCL-C (civilian) asks about symptoms in relation to "stressful experiences," and can be used with any population. The symptoms endorsed may not be specific to just one event, which can be helpful when assessing survivors who have symptoms due to multiple events.
- The Structured Clinical Interview for DSMIV[63] will be used to establish psychiatric diagnosis. The SCID is the most widely used instrument for establishment of psychiatric diagnosis.
- The Addiction Severity Index (ASI) interview is used to assess lifetime alcohol abuse. The ASI evaluates the total number of years an individual has abused alcohol in their lifetime (i.e. drinking to the point of intoxication, three or more drinks per day, on a regular basis, three or more days in a week).[66]
- Subjects will also be assessed for smoking status by using standard questions as established by Cornoni-Huntley et al.[67]
- The Hamilton Depression Scale (HAMD)[68] is a standardized scale that provides a continuous measure of depressive symptom level which will be administered to study participants.
- The Hamilton Anxiety Scale is a validated measure of anxiety symptoms with provides a continuous measure of anxiety.[69] The Ham-A will be used to assess level of anxiety in subjects
- The State-Trait Anger Expression Scale (STAXI)[70] is a self-report measure of anger expression; specifically, an "Anger-In" scale is computed by summing 8 of the items, and an "Anger-Out" scale is computed by summing the other 8 items. The STAXI has well-documented reliability and validity.[71]
- Physical activity will be assessed by means of the Baecke Questionnaire of Habitual Physical Activity.[72] This is a 16-question instrument with three sections: work, sports, and non-sports leisure activity. Questions are scored on a five-point Likert scale, ranging from “never” to “always or very often.” A modification of this questionnaire was used in the Atherosclerosis Risk in Communities (ARIC) Study.[73] The scale yields three activity subscores, work, sports and non-sports leisure indexes, which can be combined into a total score.
- The Beck Depression Inventory (BDI)[74] is a self-administered 21-item scale which has acceptable sensitivity and specificity with regards to a clinical diagnosis of depression, and also provides a continuous measure of depressive symptoms. It has been used extensively in studies of IHD.[75,76] We will also assess previous self-reported diagnosis of major depression and previous use of antidepressant medications.
- The Cook-Medley Hostility Scale (CMHS)[77] is a 50-item, true or false self-report hostility scale. It is one of the most widely used self-report hostility measures and taps into the cognitive and affective components of anger more so than behavioral anger. This scale will be used to assess participants' propensity to experience hostility and/or anger, and has satisfactory reliability. A total score will be derived by summing the item responses, and this score will be used as a continuous control variable in subsequent analyses.
- The Subjective Units of Distress Scale (SUDS)[78] is a measure of subjective distress widely used in cognitive behavioral therapy. Subjects are asked to rate current subjective distress on a linear scale of 0 to 100 with 100 being the highest level of distress. The SUDS will be used to assess

the level of stress attained in the stress challenges in addition to the visual analog scale, to verify that the procedure is stressful for the subjects.

- Analog ratings of specific mood states (Tense, Sad, Happy, Tired, Angry, Energetic and Relaxed) will also be performed to assess emotional state at the time of each of the scans.
- Subject comfort ratings will be taken during the PET portion of the study using the PASS scale.
- PTSD symptoms may be rated during the PET portion of the study using a version of the PTSD symptom scale.
- We will also administer the Pittsburgh Sleep Quality Index, a self-rated questionnaire which assesses sleep quality and disturbances over a 1-month time interval.[79]
- The Perceived Stress Scale (PSS) assesses different areas of life stress (eg, overall stress, financial stress, occupational stress, significant other stress, parental stress, and stress within friendships). Moreover, the PSS has documented validity and reliability, and is short (14 items) and easy to administer.[80] A total score is derived by summing responses to all items, with higher scores indicating greater perceived stress.
- The Clinician Administered PTSD Scale (CAPS) is a reliable and valid measure of PTSD symptomatology that provides continuous measures of symptom severity and frequency.[81] The CAPS will be one of the measures used to establish PTSD diagnosis.
- The Clinician Administered Dissociative States Scale (CADSS) is a 28-item measure of current "state" level of dissociative symptomatology. The CADSS was developed by the applicant, and there is currently a manuscript in press reporting reliability and validity, sensitivity to change, and other psychometric properties.[82]
- The Early Trauma Inventory is 56-item instrument for measurement of childhood traumatic experiences developed by the applicant and colleagues which has been shown to be reliable and valid.[83] All subjects will be assessed with both the ETI for events before the age of 18 and the Lifetime Trauma Inventory (LTI) for events after the age of 18. The ETI assesses physical, emotional, and sexual abuse, and general traumatic events in childhood, while the LTI assesses a broad range of adult traumatic events. Severity scores for individual trauma domains and composite trauma severity scores are calculated.
- All subjects will be administered four tests of the Wechsler Adult Intelligence Scale (WAIS-R) including Arithmetic, Vocabulary, Picture Arrangement, and Block Design in order to estimate an intellectual level for each subject (IQ).
- Two subtests of the Wechsler Memory Scale (WMS) will be administered according to the Russell revision.[84] The WMS-Logical Component provides measures of immediate and delayed recall of verbal material (a paragraph), with calculation of a percent retention score as the percentage relationship of material recalled at the delay in relation to immediately recalled material. The Figural Component of the WMS provides a similar measure of visual memory.
- The Revised 12-item Cognitive and Affective Mindfulness Scale (CAMS-R) is an uni-dimensional, 12-item inventory that measures mindfulness during general daily occurrences on four components allegedly needed to reach a mindful state (i.e., attention, awareness, present-focus, and acceptance/nonjudgment).
- We will measure social and emotional support by means of the Enhancing Recovery in Coronary Heart Disease (ENRICHD) Social Support Inventory (ESSI),[85] a five-item scale assessing both social and emotional support.
- We will administer the visuo-spatial and word-based N-Back tool during baseline and follow up visits. [86]

- The Toronto Alexithymia Scale (TAS-20)[87] is a 20-item instrument that is one of the most commonly used measures of alexithymia. Alexithymia refers to people who have trouble identifying and describing emotions and who tend to minimize emotional experience and focus attention externally.
- The Big Five Questionnaire (BFQ)[88] is a questionnaire to assess the five factor theory of personality.
- A credibility/expectancy questionnaire will be asked during the optional study.
- The Clinical Global Impression (CGI) scale will be used to assess illness severity and improvement.[89]

The effort will be split across two Phases, each of which will be 12 months in duration. The main objective of the first phase will be to significantly advance the mechanistic understanding of VNS efficacy in inducing psychophysiological resilience, including the effects on brain function, as well as the downstream effects on peripheral, inflammatory and other biomarkers, and physiological function (in particular, autonomic reactivity). These scientific and technological advances resulting from Phase 1 efforts will then be applied to a population of PTSD patients in Phase 2 with the aim of creating a foundation for enabling disruptive advances in the treatment of PTSD.

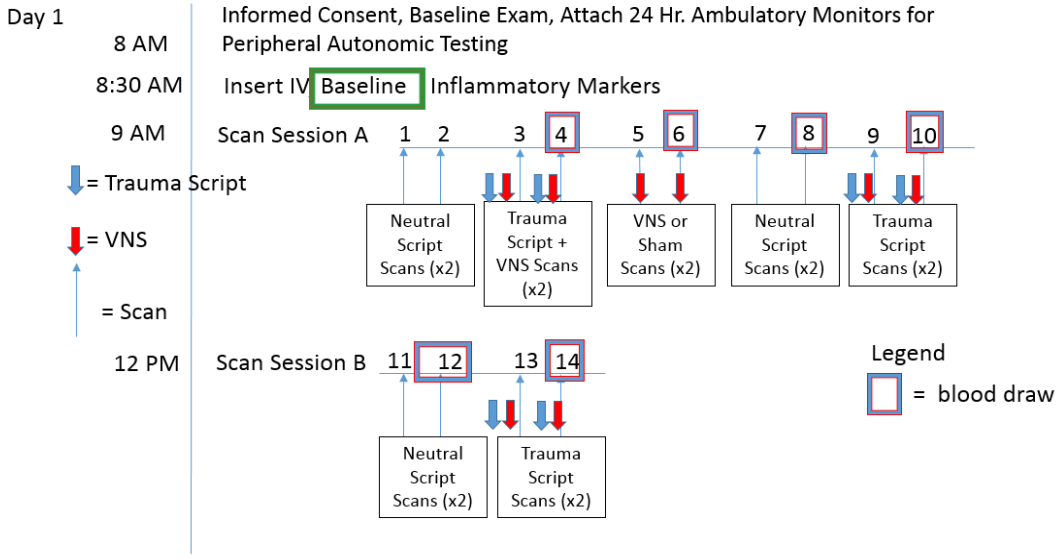
Table 1. Summary of Key Blood Biomarkers to be tested before, during, and after Emotional Stress Testing (see Budget Justification for complete list).

Blood biomarkers
Tryptophan
Kynurenine
Kynurenic acid
3-3-hydroxykynurenine
Anthranilic acid
TNF-alpha
Interferon-Gamma
Interleukin-(1Beta, 2, 4, 6, 8, 10, 12p70, 1
Macrophage migration inhibitory factor
High-mobility group protein B1
Adrenocortitropic Hormone
Cortisol
Epinephrine
Dopamine
Norepinephrine

Using the same techniques that our team has employed in several previous studies, in phase 1, we will recruit from the general outpatient population at Emory and enroll a total of 40 subjects aged 18-70 years with prior life trauma exposure, but not PTSD, to participate in a short-term (24 hour) study as described in Figure 1 as well as two additional days of VNS testing. Each subject will provide written consent, after which they will undergo a baseline psychological and health assessment. The subject will then be outfitted with ambulatory monitors to measure multiple peripheral physiological signals representative of cardiac electrophysiology (i.e., heart rate variability), hemodynamics (i.e., continuous non-invasive blood pressure, cardiac output via impedance cardiography), respiration, skin conductance, and actigraphy to account for physical activity-based changes. An electroencephalogram (EEG) measurement will be taken to assess the P3 event-related potential – a non-invasive biomarker for assessing the efficacy of VNS. Blood will be drawn as described below in Figure 1. Blood will be drawn at each time point (baseline, after the 4th, 6th, 8th, and 10th scan and again in session B after the 12th and 14th scans). A total of around 194.5ml blood or around 13 Tablespoons. A comprehensive panel of serum catecholamine and inflammatory biomarkers (see Table 2 and Budget Justification) will be obtained to examine afferent/efferent neurologic, endocrine, and immune effects.

We will perform a multiplex assay that measures both pro-inflammatory and anti-inflammatory cytokines as well as key T-helper cell TH1 and TH2 type cytokines.

Figure 1. Timeline depiction of the brain imaging study assessing the potency and kinetics of VNS in the context of stressful scripts.



- We will also examine metabolites in the tryptophan- kynurenine pathway, which are associated with inflammation and depressive symptoms, and are modulated by the VNS efferent pathway [90]. Symptoms will be assessed via the PTSD checklist scale, subjective units of distress scale (SUDS), and fear and anxiety analogue scales at multiple time points before and after the stressor. Potential adverse effects of VNS will also be queried, including: voice hoarseness, throat pain, cough, dysphagia, abdominal pain, headache, and shortness of breath. Recently, electroCore reported on the effects of non-invasive VNS on autonomic and inflammatory parameters in healthy humans. Reduction of cytokine levels (biomarkers of inflammation) appeared most evident in subjects who had elevated levels at baseline, suggesting that non-invasive VNS may have a role as an anti-inflammatory intervention acting through VNS of the cholinergic anti-inflammatory pathway [91]. This study will go a step further to correlate the brain and peripheral changes that occur after VNS stimulation with the subjective improvements and EEG metrics of efficacy. These correlations

may allow us to validate whether the effects are due to vagal stimulation by itself, or other potential mechanisms.

- Our team has significant expertise in developing such mental stress challenges, and have demonstrated the effectiveness of such methods in multiple studies involving populations such as PTSD patients [92] (see Figure 2). These acute mental stress challenges mimic similar brain responses as one would observe from a PTSD patient exposed to a traumatic reminder. Understanding how VNS can potentially improve the resilience of the brain to traumatic reminders is the key element that can allow this technology to succeed in reducing symptoms in PTSD patients. A total of eight HR-PET conditions will be obtained in the first series, as shown in Figure 1. The protocol follows established methods [93-95] which have been found to effectively elicit trauma-related stress responses (Figure 2). Prior to the stress procedure, the subject will prepare two scripts of the most stressful events s/he experienced. The scripts will be transcribed, edited to last 120 seconds each, and recorded by a research associate in a normal voice, in the first person, present tense. After initial set up and rest period subjects will rest for 30 min. Next, subjects will undergo a series of listening exercises while lying in the HR PET scanner with physiologic monitors attached. After an initial baseline period of resting (condition 1), the subjects will then listen to two neutral 120 second scripts (condition 2), followed by a playback of two trauma scripts (condition 3). The subject will be instructed to image each event as vividly as possible. VNS will be given immediately at the end of all traumatic scripts and stressors. For scans five and six, we are looking at effects of VNS on the brain with no intervention. The subjects will undergo VNS vs. Sham VNS stimulation as per published methods [45] (see above) and undergo scanning for condition 4. The SUDS [78] and other behavioral measures are assessed in the beginning and after each task to assess the efficacy of the trauma recall task in eliciting stress. A similar protocol will be repeated around 12 PM (i.e, baseline assessment and with exposure to neutral and traumatic scripts, or Scan Session B) in conjunction with HR-PET measurement and measurement of biomarkers and physiology. Patients will stay at the Emory University Center for Systems Imaging while they are undergoing detailed autonomic monitoring and sampling of blood biomarkers. The blood draws will occur prior to stress, various time points during stress, and post-stress.

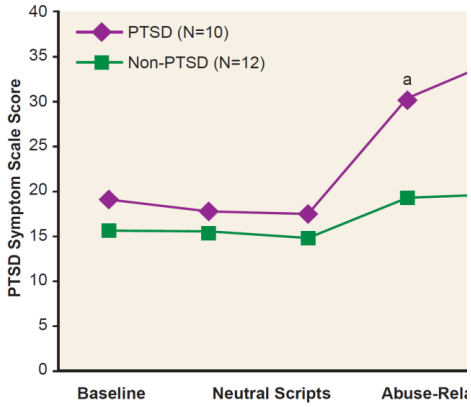


Figure 2. PTSD Symptom Scale Scores for women with and without PTSD during baseline and during readings of neutral scripts and scripts related to their sexual abuse during childhood. * indicates that there was a significant increase in PTSD symptoms during the traumatic scripts in women with PTSD relative to women without PTSD (group-by-time interaction, F=7.88, df=4, p<0.001). [19]

- Accordingly, our initial analysis will focus on the imaging data. We will first assess whether, in all patients, the expected direct effects of VNS are observed: specifically, we will assess the direction of changes in perfusion at the amygdala (decrease expected), dorsal anterior cingular cortex (dACC, increase expected), and medial prefrontal cortex (mPFC, increase expected). Second, we will quantify these increased / decreased perfusion levels at the maximal / minimal values across all subjects, and determine whether statistically significant changes are observed in VNS vs. sham conditions. Third, we will determine the time delay following VNS delivery at

which significant changes are observed for 90% of the subjects. Fourth, we will examine whether the changes in perfusion associated with acute mental stress are diminished with VNS delivery, as we hypothesize. Fifth, we will map the kinetics of the VNS delivery, by finding for each patient the changes in mental stress response over time

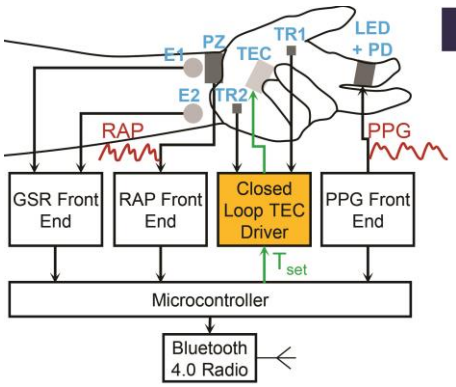
- VNS administration: The "VNS" group will be administered VNS using the electroCore GammaCore-S non-invasive VNS device. The intensity of the stimulus (i.e. the current amplitude) will be adjusted by the user, to the maximum tolerable level to ensure VNS without causing excessive pain (typically 10-30 V), the burst frequency to 5 kHz, and the envelope frequency to 25 Hz. These are the standard frequency settings that electroCore has demonstrated to be most effective in capturing the vagus nerve based on evoked potential studies. The duration of delivery will be 2 minutes, and the beginning will coincide with initiation of acquisition of the HR-PET scan which will be 90 seconds in duration; following an additional 8 minutes, a second VNS delivery will be administered, in conjunction with which another scan will be obtained. Each subject in the "SHAM" group will undergo the action of administering the intervention, but the device will be programmed such that no actual power is being delivered. The stimulators provided by electroCore (see support letter) will include analog trigger outputs that can provide timing information on when the stimulus is being delivered; these trigger outputs will then be used in conjunction with EEG signals measured from the subjects to ensure that the P3 evoked potential is observed. If not observed, the device will be repositioned.
- During their visit, participants may be outfitted with an activity tracker bracelet (Phillips Actiwatch or equivalent) for the purposes of monitoring their physical activity and sleep over the course of around 2 weeks. The subjects may be asked to complete a few sleep, activity, and/or mood assessments during this time. The activity tracker bracelet will be returned directly to Emory research staff at the last visit. The tracking bracelet will not have any personally identifying information downloaded on it, and the data will be transferred to the secure research drive for sleep staging and physical activity analysis. The watch may also have a mood survey component for subjects to rate their moods twice daily.
- The data collected in Task 2 will include: (1) HR-PET images taken at multiple time points, both before and after the acute mental stress challenge, and both with and without VNS delivery; (2) peripheral physiological measurements (e.g. ECG, PPG, ICG, GSR, SCG etc.) and associated signal features (e.g. heart rate, heart rate variability, pulse arrival time, pulse transit time, etc.); and (3) blood biomarkers (e.g. catecholamine levels, cytokines, etc.). The anticipated changes in physiological signal (2) and serum (3) biomarkers are predicted to occur after the changes in imaging data, since they are downstream (secondary) effects of VNS.
- The second thrust of the analysis will focus on linking brain function changes associated with acute mental stress to downstream changes in parameters measured from (2) and (3). The goal will be to, then, create a predictive model for inputting the downstream measured data, and predicting the onset of a stressful episode. A secondary goal will be to predict the optimal timing for another VNS delivery based on these peripheral measurements. We will first quantify which parameters in these two sets of data change significantly with VNS delivery. Second, we will determine the temporal order in which the changes occur, and examine whether certain association rules (e.g. increased perfusion in the mFPC leads to increased heart rate variability) accurately fit the measured data. Third, we will develop initial predictive models, using neural networks and support

vector machines, to use the salient features from (2) only, (3) only, and (2) and (3) together, to predict the onset of an acute mental stressor, and quantify the sensitivity and specificity of this prediction for the subject population. Fourth, we will develop a model for quantifying the efficacy of VNS over time based on these peripheral measurements, such that – when the VNS efficacy has decreased and stress resilience is compromised – a second delivery of VNS can be suggested to the user.

- At the end of the lab component of the study (day 3 of participation), we will perform a specific validation of the Gammacore device and compare it to another non-invasive VNS device with recent efficacy in clinical trials, the Parasym, which stimulates the vagus nerve through electrical stimulation of the tragus (ear). This will include approximately 1 hour testing that involves physiologic assessment of the Gammacore device (VNS vs. Sham), followed by a 15 minute break, and then another stimulation by the Parasym device for 15 minutes. Finally there will be a 15 minute recovery period. The assignment of active vs. sham VNS devices will be random but consistently the same for both Gammacore and Parasym. Sham stimulation may include a low-voltage stimulation in a nearby area (for example, sternocleidomastoid area for Gammacore, and earlobe for Parasym device) to accurately measure EEG in response to VNS.

Developing Active Sensing Technologies for Assessing VNS Efficacy using Peripheral Physiological Biomarkers

- Although studies of vagal nerve stimulation show no gross changes in heart rate or ECG, stimulation of the autonomic nervous system likely has physiologic effects beyond those parameters. In order to assess the role of various feedback mechanisms, we propose the development of novel methods to test the homeostasis mechanisms related to temperature, which likely have a basis in the hypothalamus. Such models are therefore likely to more clearly show the effects of stress and VNS on neurobiology. More specifically, we believe temperature-based perturbations are likely to better uncover hypothalamic dysfunction during stress, and improvements with VNS, as opposed to only measuring such metrics at rest.
- We plan to refine our existing active, physiological modulation and monitoring prototyping comprising: (1) a thermoelectric cooler for increasing and decreasing palmar skin temperature, (2) optical and mechanical sensors for measuring the ensuing changes in blood volume pulse in response to skin temperature stimulation, and (3) galvanic skin response (GSR) sensing to examine the skin conductivity changes associated with the sweat gland activation in response to the stimulation. By pursuing an active sensing approach, where the physiology is perturbed and the neuro-vascular responses are recorded, we posit that more robust measurements of autonomic reactivity can be obtained in ambulant PTSD patients than with any existing passive monitoring technologies. Our hypothesis for this new sensing approach is that the thermal reflexes will both become slower (i.e. more time delay between ΔT_{sk} and the ensuing blood volume pulse or GSR



Sensor / Actuator	Transduced Signal	Health Param
E1 and E2 (Electrodes)	Galvanic Skin Response (GSR)	Opening of S Glands
PZ (Piezo-Electric Film)	Radial Artery Pulse (RAP)	Radial Pul Waveforr
TEC (Thermo-Electric Cooler)	Heating and Cooling	N/A
TR1 and TR2 (Thermistors)	Skin Temperature	Palmar Tempe
LED + PD (LED and Photodiode)	Photo-plethysmogram (PPG)	Blood Volume

Figure 3. Block diagram of glove hardware concept and associated signals and health parameters that can be measured statically. Importantly, these parameters will also be measured in response to changes in skin temperature delivered by the thermo-electric cooler (TEC).

changes) and less pronounced in PTSD patients with increased sympathetic tone (and associated symptom burden).

- The block diagram of the sensors and electronics, with associated transduced signal and health parameters is shown in Figure 3. We plan to refine the existing bench-top hardware in Dr. Inan's lab to systematically elucidate the effects of multiple heating and cooling protocols on the blood volume pulse and GSR signals. These protocols will include the modulation of skin temperature at one palm, and measurement of the physiological parameters at both the modulated and contralateral hands; the modulation of both palms with the same protocol and measurement from both hands; the modulation of both hands with asymmetric protocols (e.g. heating the left while cooling the right hand) and measurement from both hands. The goal of these studies will be to optimize the ability to accurately assess autonomic reactivity in PTSD patients. To the best of our knowledge, these innovative concepts of complex neuro-vascular modulation through thermal energy have not been explored in PTSD patients, and hold great promise for providing in-depth autonomic reactivity assessment even in the presence of movement artifacts or other confounding variables such as environmental changes (e.g. ambient temperature).

Kinetics of VNS on Peripheral Autonomic Function for Two Days

- This task will test the longer-term kinetics of VNS on peripheral autonomic function, but extends the time period to 2 additional days and focuses on peripheral markers with the goal of creating a closed-loop system based on peripheral physiology. Subjects will undergo a 2-day study in which they are assigned to either a fixed schedule of daily VNS twice a day therapy (group A, n=20) or a sham exposure to VNS (group B, n=20). Peripheral physiological and serum biomarker measurements as described in Base Task 2 will be measured, along with measures of subjective stress using the SUDS scale and fear and anxiety analogue scale. During this time, each subject will wear ambulatory, comprehensive physiological monitoring systems that will measure a range of signals related to autonomic function. Stressful stimulations will be performed twice per day at random intervals to induce external stress and test the efficacy of VNS. Blood will be drawn twice on Day1 and Day2 (57cc each) using venipuncture or an indwelling catheter for analysis, and the subject will use the active sensing hardware designed as part of Base Task 3. During the subject's stay, several acute mental stressors will be introduced without warning. This may include a speech

task, a math challenge, a cognitive challenge, a difficult encounter with an actor, and/or a trauma reminder. This will also ensure adequate testing of the interventions and mimic stressful episodes in subjects with PTSD.

Assessments and Procedures (Longitudinal Study)

- A subgroup of the Phase 2/PTSD patients (n=18) will be offered a longitudinal assessment of VNS or sham. Subjects will be sent home with a pre-addressed FedEx package (to return devices if needed), ballistocardiography (BCG) scale, Garmin VivoSmart device, an ECG patch, recorded traumatic scripts, an MP3 player (if needed), and VNS/sham device.
- VNS or sham will be self-administered with the protocol above 4 times a day for three months. Subjects will be asked to listen to a 60 second recording of their traumatic script in conjunction with the application of VNS or sham. The study team will attempt to video conference up to twice weekly and meet monthly with the subjects.
- At home, subjects will be asked to do a 1-2 minute ballistocardiography (BCG) exam (stand on scale and hold handlebars) before and after administration of VNS/Sham (6 daily measurements). They may also be fitted with an activity tracker bracelet to wear for one week after each of their visits. Some participants may be asked to wear the bracelet for the entire study duration. They might be asked to pair the bracelet with their phone. There is a risk of data breach in using the Garmin activity tracker. This will be listed as a risk in the informed consent and will be discussed with the subjects. The research team will manage this risk by de-identifying the data. A subject ID will be used instead of a name. Information about Garmin's Cloud/Connect service and terms will also be given to the subjects. (https://www.garmin.com/en-US/privacy/connect/policy/) (https://www.garmin.com/en-US/privacy/connect/).The participants may be asked to wear an ECG patch that measures heart rhythm. The patch will be worn on the upper chest. It is waterproof. Participants may be asked to wear it for two one week sessions. It can be mailed back in the prepaid mailer if necessary. As with the activity tracker, it will not contain or transmit any personally identifiable data.
- On day 7 and at the end of months 1 and 2, the subject will be scheduled for an in-person visit. Devices will be checked and data downloaded by study staff, we will draw blood (57cc per visit) and complete the following questionnaires: PCL, CAPS, analogue ratings of mood states, N-Back tasks, and Wechsler Memory Scale with VNS/sham at time of paragraph reading.
- One week prior to and at the end of the three months, we will schedule in-person visits. The study may ask the participant to come in 1 week prior to the end of month 3 in order to apply the ECG patch. This will be returned the following week. At the end of month 3 visit, most of the study procedures performed at baseline (including Day 1 Scan Session A) will be repeated. Blood will be drawn using the same timing as baseline. A total of around 142.5ml or 9.5 Tablespoons will be drawn at this visit. We will complete the following questionnaires: PCL, CAPS, analogue ratings of mood states, N-back tasks, and Wechsler Memory Scale with VNS/sham at time of paragraph reading.
- All subjects may then be offered to self-administer active VNS for an additional three months, following which we will schedule an in-person visit. During this visit, we will draw blood (57cc) and complete the following questionnaires: PCL, CAPS, analogue ratings of mood states, N-Back tasks, and Wechsler Memory Scale with VNS/sham at time of paragraph reading.

Image Processing and Analysis Subjects will be scanned on a High Resolution Research Tomograph (HRRT) (CTI, Knoxville TN) (2 mm resolution). The HRRT has 2 mm spatial resolution and 10-fold better sensitivity than other currently available PET cameras [96,97]. Each subject will undergo positron emission tomography (PET) and O-15 water scanning of the brain in conjunction with VNS and traumatic script readings. $H_2[^{15}O]$ will be prepared on-site in the Emory PET Center cyclotron. During the hours of the test, subjects will recline in bed except to use the bathroom. An intravenous infusion of normal saline will be started by a physician, nurse or technologist to permit the bolus injection of $H_2[^{15}O]$. Subjects will be scanned with eyes open in a dimly lit room on the HRRT. The subject will be placed in the scanner with the head held in a head holder and the head will positioned with the cantho-meatal line parallel to the external laser light. Subjects will receive a 20 mCi intravenous bolus of $H_2[^{15}O]$ for each of the 14 scans. Subjects will be scanned during exposure to neutral and traumatic scripts. Order of traumatic and neutral scripts will be fixed. Each scan will last 90 seconds, with the PET scan acquisition beginning at the initiation of the condition. If the PET scan imaging is not available, brain scans will not be performed. Subjects will be made aware of this (no debriefing will be needed). There are less risks to the subject since they will not have brain scans.

PET images are realigned to the first image in the scanning session using statistical parametric mapping (spm). Images are then transformed into a common neuroanatomical space, smoothed, and subjected to statistical analysis using methods previously described by us in detail [98,99] Significance is defined as a minimal cluster of 11 voxels with a $p<0.005$ in hypothesized areas (amygdala, medial prefrontal cortex). This method minimizes Type I and Type II error.[100] Comparison regions will include motor cortex, sensory cortex, and cerebellum.

Statistical Considerations:

We will use analysis of variance models with change in blood flow as dependent variable and absolute blood flow during control task as well as study group as explanatory variables. This analysis will assess differences in brain function according to diagnosis correcting for potential differences in baseline (control task) values. The ANOVA model will be blocked by the matching factor of past alcohol exposure. Analyses will be adjusted for whole brain blood flow. Additional control factors, including patients' age, age of onset of traumatization, years of education, IQ, PTSD symptom level and duration of disease, use of oral contraceptive birth control, and smoking, will be entered in the model if they are significantly different between the study groups at an alpha level of 0.25, and/or if they are found to be confounders in the analysis, i.e., if they cause a change of >10% in the parameter estimate for study group when entered in the model.[101] Adjusted means of blood flow will be computed for each combination of group and task to better describe the results. Point estimates and confidence intervals for the difference between groups will be assessed before and after including the covariables mentioned above in the model. Additional analyses will compare PTSD women with and without a past history of alcohol or substance abuse/dependence, and with and without a history of co-morbid depression.

In all these analyses linear model assumptions, adequacy of fit, and presence of outliers will be checked by inspecting residual plots and influence statistics. Collinearity for variables included in the model will also be checked. Appropriate remedies will be applied, if necessary, to improve model fit and minimize collinearity.[102]

Additional exploratory analyses will use subtraction analysis techniques to look for additional areas of activation for the purpose of generating hypotheses for future studies. Images of absolute blood flow will be subjected to a subtraction type analysis. Images will be realigned to the first image in the series and warped into a common anatomical space using methods described in the preliminary results. Images will be then analyzed using the general linear model of Friston for statistical analysis.[103,104] The mean of blood flow scans within condition will be calculated and comparisons performed between conditions to assess blood flow with the condition. Areas of increased and decreased blood flow within patient group will be examined, as well as the interaction between condition and diagnostic group. Images of t statistic will be created and surveyed for hypothesized regions (i.e. amygdala, medial prefrontal cortex) where uncorrected z score values are greater than 2.86 (corresponding to a corrected p value of > 0.005).

Calculation of Sample Size

- For calculation of sample size needed for PET measurement of amygdala activation, we used values for blood flow in the amygdala (scaled to whole brain blood flow) based on previous pilot data. Fear-related tasks resulted in a 6.9 $\pm$ 4.8% increase in amygdala blood flow versus 1.2 $\pm$ 1.4% in controls (effect size 1.39). With two tails, an alpha value set at 0.05 and a power of 0.80, 13 subjects are needed in each group (VNS v sham) to show a significant difference between patients and controls (i.e. group by condition interaction).The number of subjects in each group will therefore be adequate to answer the questions proposed in this study.
- During extinction PTSD patients in a pilot study of fear had a -10.7 $\pm$ 1.9% decrease in blood flow in the medial prefrontal cortex with no change in the controls (effect size 5.33). With two tails, an alpha value set at 0.05 and a power of 0.80, 13 subjects in each group will be enough to show a significant difference between VNS stimulated and sham stimulated controls (i.e. group by condition interaction). We therefore have an adequate sample to answer the questions proposed in this study.
- With two tails and an alpha value set at 0.05, a correlation of 0.432 in 21 subjects will be required to show a statistically significant relationship between skin conductance responses and amygdala and medial prefrontal function measured with PET. Thirty subjects in the PTSD group will therefore be adequate to answer the questions proposed in this study.

Validation of nVNS through Modelling:

In a sub-sample of four subjects who have gone through active VNS after their completion of the primary protocol we will invite them to participate in an additional study which will serve to validate nVNS. Subjects will undergo a structural magnetic resonance imaging (MRI) and computed tomography (CT) scan of the neck. This information will be used for finite element modelling in order to create a three dimensional image of the vagus nerve and the tissues that modulate transmission of electrical signal after application of nVNS to the neck. No further testing, however, will be required. Subjects will be paid $75 for the MRI and $75 for the CT. For the magnetic resonance imaging (MRI) studies, subjects will undergo structural imaging of the brain at rest.

The MRI sequences parameters are shown in table 2 below. Briefly, high resolution isotropic images will be collected without contrast including a constructive interference in steady-stage (CISS), 3D T2-weighted short-tau inversion recovery (STIR) SPACE (sampling Perfection with Application optimized contrasts using different flip angle Evolution) and volumetric interpolated breath-hold examination (VIBE). The field-of-view (FOV) will extend from the entire hippocampus and frontal cortex and extending inferior to the lower neck.

Table 2. Parameters of high resolution skull base MRI imaging adapted from Wen et al. Magn Reson Imaging Clin N. Am 26 (2018) 101-109 (PMID: 29127999) .

	TR (ms)	TE (ms)	TI (ms)	Flip angle (degree)	FOV (mm)	Slices	Voxel Size (mm)	NEX	Duration
STIR SPACE	3000	256	220	-	256	~88	1.0x1.0x1.0	1.4	5:23

VIBE	4.93	2.46	-	9	210	~112	0.8x0.8x0.8	3	4:38
CISS	5.46	2.43	-	42	152	~112	0.6x0.6x0.6	1	5:17
MPRAGE	2530	1100	-	8	256	~190	1.0x1.0x1.0	1	9:00

For the CT scan subjects will lay supine on the CT couch and undergo high resolution imaging of the head and neck. Images will be collected in helical mode at 120kVp with automatic tube modulation (100 < mA < 600). Images will be reconstructed with an iterative routine at a slice thickness and interval of 1.25mm.

HUMAN SUBJECTS

Subject population:

Phase 1 - Forty men and women age 18-70 with a history of exposure to psychological trauma and no PTSD. Subjects will be recruited from newspaper advertisements, online websites, and/or fliers.

Phase 2 – Forty men and women age 18-70 with PTSD. Subjects recruited from newspaper advertisements, online websites, and/or fliers.

Longitudinal Study – 18 men and women age 18-70 with PTSD. Subjects will be participants in our Phase 2.

Risks:

Psychological Assessments There is a risk that patients may become more upset as a result of being administered questionnaires or assessments related to their history of trauma. If this occurs they will be assessed by Dr Bremner, a board-certified psychiatrist with extensive experience in the clinical treatment of PTSD.

Intravenous Catheters and Venipuncture Placement of an intravenous catheter can result in infection, bruising of the skin, or a blood clot in the vein. These complications are not common when done by a professional under clean conditions. Dizziness and fainting are rare risks of blood draws. There are no long-term side effects.

Vagal Nerve Stimulation Stimulation of the vagus nerve may cause muscle twitching, discomfort, or pain during treatment. Hoarseness, a change in voice, or change in taste are other temporary possible consequences but these resolve when treatment is stopped.

H2[15O] and PET H2[15O] or radiolabelled water has been administered to thousands of subjects without side effects or toxicity to date at the doses to be used in this study. Dr Bremner holds an IND for use of radiolabelled water at Emory University. H2[15O] is a radioactive materials. Each patient will have fourteen H2[15O] blood flow scans in two sessions. For each of the fourteen O-15 scans 20 mCi of H2[15O] will be injected as an intravenous bolus. The body organs which receive the highest dose of H2[15O] is the intestine and gonads. The radiation dose to body organs in this study is well within the Food and Drug Administration (FDA) national guidelines for radiation exposure for human research studies and less than the total amount that is permitted for research studies in one year. Subjects will be asked if they have participated in research studies that involved radiation over the past year. Also, they will be advised that they should not participate in other research studies involving radioactivity over the next year without consulting Dr. Bremner first. There is a small risk of claustrophobia with PET. If needed the study will be stopped.

Stressful Tasks: Listening to traumatic scripts, giving public speeches, or being exposed to random stressors may be associated with a feeling of upsettedness or increase in mental symptoms. The

research staff will debrief subjects, which involves talking about the experiences and helping subjects to process whatever emotions come up for the subject. If needed, for example if the subject is experiencing suicidal thoughts, further action will be taken if necessary. For example, if subjects are suicidal they will be escorted to the Emergency Room, and they will be instructed that if they have problems with feeling upset they should call one of the clinicians and if they develop active suicidal ideation with a plan they should go to the Emergency Room.

MRI and CT scanning: There is the risk that if subjects have indwelling metallic foreign bodies in the head that MRI scanning may cause local tissue damage. All subjects will be screened for indwelling foreign bodies and individuals with indwelling bodies will be excluded. Some individuals may feel anxiety while in the scanner. If needed, lorazepam 1-2 mg will be administered for anxiety. We have found this to be an effective method for managing anxiety associated with scanning. There are no other risks of MRI scanning. CT imaging is associated with a small amount of radiation exposure. Dr. Bremner will review the subjects to ensure that there is not an excess in radiation exposure for all research imaging performed with the current scan and the prior 12 month period per guidelines.

CT imaging of the head and neck involves exposure to radiation from the CT scans. CT imaging is used routinely for medical care. The estimated radiation dose that subjects will receive is equal to or less than the annual radiation exposure limit allowed for persons who are occupationally exposed to radiation (for example, x-ray technologist, radiologist). The principal risk associated with a radiation dose is the possibility of developing a radiation-induced cancer later in life. The risk for radiation-induced cancer from this study is minimal.

Procedure for Protecting Against Potential Risks: Screening: Supervision: All of the scans will be done in the presence of constant supervision with experienced research staff always in attendance and in an institution specifically designed, equipped and functioning in support of these type studies. In the event that some serious medical complications could occur, the PET scan facilities are located in Wesley Woods Hospital on the Emory University campus and can provide immediate care. *Confidentiality:* All of the information obtained from patients and healthy subjects is quoted by number and kept locked in confidential files. This information is available to study investigators. *Risk-Benefit Ratio:* The risks of participation in this study are small. These are outweighed by the potential benefit which a better understanding of the effects of PTSD could have for society.

Adverse events (AEs) are evaluated at each visit and reported to IRB within 14 days. Serious adverse events (SAEs) (as defined by CRF 312.32) are reported to the IRB within 24 hours of an investigator being informed. All AE and SAE reports will include a description of the event, severity, relationship to the study medication or procedures, any intervention required, and date of the resolution of the event (or otherwise listed as ongoing). SAEs include any fatal event, immediately life threatening event, permanently or significantly disabling event, event requiring prolonged inpatient hospitalization, or any congenital anomaly, and any other event that deems to result in a significant hazard, contraindication, side effect or precaution.

All non-compliance/unanticipated problems, serious adverse events, audits and investigation reports will be reported to the Department of Defense (DoD) Human Research Protection Program (HRPP) and SPAWAR Systems Center Pacific (SSC Pacific).

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