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## Transcutaneous Cervical Vagal Nerve Stimulation in Patients with Posttraumatic Stress Disorder (PTSD): A Pilot Study of Effects on PTSD Symptoms and Interleukin-6 Response to Stress

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### Abstract

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Supplementary materials

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**Background:** Posttraumatic stress disorder (PTSD) is a highly disabling condition associated with alterations in multiple neurobiological systems, including increases in inflammatory and sympathetic function, responsible for maintenance of symptoms. Treatment options including medications and psychotherapies have limitations. We previously showed that transcutaneous Vagus Nerve Stimulation (tcVNS) blocks inflammatory (interleukin (IL)-6) responses to stress in PTSD. The purpose of this study was to assess the effects of tcVNS on PTSD symptoms and inflammatory responses to stress.

**Methods:** Twenty patients with PTSD were randomized to double blind active tcVNS (N=9) or sham (N=11) stimulation in conjunction with exposure to personalized traumatic scripts immediately followed by active or sham tcVNS and measurement of IL-6 and other biomarkers of inflammation. Patients then self administered active or sham tcVNS twice daily for three months. PTSD symptoms were measured with the PTSD Checklist (PCL) and the Clinician Administered PTSD Scale (CAPS), clinical improvement with the Clinical Global Index (CGI) and anxiety with the Hamilton Anxiety Scale (Ham-A) at baseline and one-month intervals followed by a repeat of measurement of biomarkers with traumatic scripts. After three months patients self treated with twice daily open label active tcVNS for another three months followed by assessment with the CGI.

**Results:** Traumatic scripts increased IL-6 in PTSD patients, an effect that was blocked by tcVNS ( $p<.05$ ). Active tcVNS treatment for three months resulted in a 31% greater reduction in PTSD symptoms compared to sham treatment as measured by the PCL ( $p=0.013$ ) as well as hyperarousal symptoms and somatic anxiety measured with the Ham-A  $p<0.05$ ). IL-6 increased from baseline in sham but not tcVNS. Open label tcVNS resulted in improvements measured with the CGI compared to the sham treatment period  $p<0.05$ ).

**Conclusions:** These preliminary results suggest that tcVNS reduces inflammatory responses to stress, which may in part underlie beneficial effects on PTSD symptoms.

## 1. Introduction

Posttraumatic Stress Disorder (PTSD) is a disabling disorder that affects the quality of life and productivity of millions of Americans (Bremner, 2016). The standard of care for PTSD includes psychotherapy and/or medication (Ballenger et al., 2000; Foa et al., 1999; Foa et al., 2007; Foa and Rothbaum, 1998; Hembree et al., 2003; Lancaster et al., 2016; Schnurr et al., 2007), however current treatments are characterized by high rates of non-completion and/or limitations in efficacy (Ballenger et al., 2004; Davis et al., 2016; Hembree et al., 2003; Schottenbauer et al., 2008). Some reports concluded that there is insufficient evidence to conclude that first line medication treatment with Selective Serotonin Reuptake Inhibitors (SSRIs) are effective for PTSD (Institute of Medicine of the National Academies, 2014). New approaches to treatment are needed, especially those that target the underlying psychobiology of PTSD, involving core changes in brain and autonomic nervous system (Reinertsen et al., 2017; Shah et al., 2013) and immune function (Neigh and Ali, 2016), that maintain symptoms of the disorder (Bremner, 2016; Shah et al., 2013).

Neuromodulation is a new approach that may be particularly useful in addressing the underlying psychobiology of stress-related psychiatric disorders (Adair et al., 2020; Bikson

et al., 2016; Bikson et al., 2017b; Bremner et al., 2020b; Krames et al., 2018; Schachter and Saper, 1998; Tortella et al., 2015; Woods et al., 2016). Vagal Nerve Stimulation (VNS) is a form of neuromodulation that has been shown to be efficacious in the treatment of epilepsy (Ben-Menachem et al., 1999; Ben-Menachem et al., 1994; George et al., 1994; Handforth et al., 1998; Salinsky et al., 1999; The Vagus Nerve Stimulation Study Group, 1995) and treatment-refractory major depression (Aaronson et al., 2017; Berry et al., 2013; Dell-Osso et al., 2013; George et al., 2005; George et al., 2003; George et al., 2000; Marangell et al., 2002; Rush et al., 2000; Rush et al., 2005a; Rush et al., 2005b; Sackeim et al., 2007; Sackeim et al., 2001a; Sackeim et al., 2001b). Implantable VNS devices are currently approved by the Food and Drug Administration (FDA) for treatment resistant major depression (Aaronson et al., 2017; George et al., 2003; Terry, 2014). Beneficial effects of VNS that may be particularly useful for stress-related psychiatric disorders including blocking of sympathetic (Pena et al., 2014; Peña et al., 2013; Schomer et al., 2014) and immune function (Bansal et al., 2012; Borovikova et al., 2000), and enhancement of cognition (Clark et al., 1999; Jacobs et al., 2015; Sackeim et al., 2001a; Sjögren et al., 2002; Smith et al., 2005; Sun et al., 2017; Vonck et al., 2014). Implantable devices have not been widely implemented in psychiatry, however, in part due to lack of reimbursement by Medicare and private insurers (Feldman et al., 2013).

A new generation of non-implantable VNS devices has the potential to be more widely implemented in psychiatry due to lower cost and greater convenience (Bremner and Rapaport, 2017). VNS can be applied to branches of the vagus nerve in the ear (transcutaneous auricular VNS, or taVNS) or in the neck, where it travels through the carotid sheath (transcutaneous cervical VNS, or tcVNS) (Adair et al., 2020; Badran et al., 2019; Bremner et al., 2020b). PTSD is associated with an increase in the blood concentrations of the inflammatory marker interleukin (IL)-6 at baseline (Gill et al., 2010; Gill et al., 2008; (Guo et al., 2012); Li et al., 2014; Lindqvist et al., 2017; Miller et al., 2001; Passos et al., 2015; Sutherland et al., 2003; Tucker et al., 2010; Vidovic et al., 2011; von Känel et al., 2010) and in response to mental stress such as public speaking (Lima et al., 2019) or exposure to personalized traumatic scripts (Bremner et al., 2020a) as well as in diurnal cerebrospinal fluid (CSF) (Baker et al., 2001). Studies have shown increased blood concentrations of interferon $\gamma$  (IFN $\gamma$ ) at baseline (Guo et al., 2012; Hoge et al., 2009; Lindqvist et al., 2014; Passos et al., 2015; Woods et al., 2005; Zhou et al., 2014) and in response to traumatic script stress in PTSD (Bremner et al., 2020a). Other studies showed increased baseline Tumor Necrosis Factor (TNF)- $\alpha$  blood concentrations in PTSD (Gill et al., 2010; Lindqvist et al., 2017; Lindqvist et al., 2014; Passos et al., 2015; Sutherland et al., 2003; Vidovic et al., 2011; von Känel et al., 2007). Animal studies show VNS decreases both IL-6 (Borovikova et al., 2000; Brock et al., 2017; Corsi-Zuelli et al., 2017; Das and Basu, 2008; Das, 2007, 2011; Jan et al., 2010; Li and Olshansky, 2011; Marsland et al., 2007) and TNF- $\alpha$  (Bansal et al., 2012; Jan et al., 2010; Marsland et al., 2007). Studies using both implantable devices (De Herdt et al., 2009) and tcVNS (Brock et al., 2017; Lerman et al., 2016) show VNS also decreases TNF- $\alpha$  in human subjects, while another study showed long term treatment with tcVNS lowered both TNF- $\alpha$  and IL-6 in patients with Sjögren's Syndrome (Tarn et al., 2019). We showed that tcVNS blocks IL-6 and IFN $\gamma$  response to traumatic script stress in PTSD (Bremner et al., 2020a) and blocks the rise in

Pituitary Adenylate Cyclase Activating Peptide (PACAP) over three days of stressful tasks in traumatized subjects with and without PTSD (Gurel et al., 2020c). We also previously reported that tcVNS in traumatized healthy human subjects with and without PTSD blocked peripheral sympathetic and enhanced parasympathetic responses both at baseline and in response to both personalized traumatic scripts and mental stressors (Gazi et al., 2020; Gurel et al., 2020a; Gurel et al., 2020b; Gurel et al., 2018; Gurel et al., 2020d; Gurel et al., 2020e) and modulated brain response to traumatic scripts (Wittbrodt et al., 2020), and other studies reported that tcVNS blocked sympathetic function in healthy subjects (Brock et al., 2017; Lerman et al., 2019) while taVNS blocked sympathetic function in healthy human subjects (Badran et al., 2018b; Bretherton et al., 2019; Clancy et al., 2014) and patients with co-morbid mild Traumatic Brain Injury (mTBI) and PTSD (Lamb et al., 2017). This work replicated findings in healthy subjects using implanted VNS (Schomer et al., 2014). A nonrandomized study of taVNS in patients with depression showed efficacy at four weeks for symptoms of depression when compared to sham stimulation (Rong et al., 2016). Other studies in patients with depression showed taVNS resulted in changes in brain regions implicated in that disorder (Fang et al., 2017; Fang et al., 2016; Liu et al., 2016; Tu et al., 2018; Wang et al., 2018). The purpose of the current study was to assess the efficacy of tcVNS, delivered in a longitudinal study, compared to sham stimulation for the treatment of PTSD, and to assess the effects on stress-induced inflammation. We hypothesized that tcVNS would be associated with improvement in symptoms associated with PTSD, in particular those driven by increased sympathetic function including hyperarousal and somatic anxiety, and a reduction in stress-induced IL-6 activation.

## 2. Materials and Methods

### 2.1. Human Subjects

The research reported here was approved by the Institutional Review Boards of Emory University, Georgia Institute of Technology, and the Space and Naval Warfare Systems Command (SPAWAR) Systems Center of the Pacific and the Department of Navy Human Research Protection Program. Patients were studied between February 2019 and March 2020 at the Emory University School of Medicine. Subjects provided written, informed consent for participation. Subjects included physically healthy adults age 18–70 with a history of psychological trauma and the current diagnosis of posttraumatic stress disorder (PTSD) (Figure 1). Subjects were excluded with the diagnosis of schizophrenia, schizoaffective disorder, bipolar disorder, bulimia or anorexia, as defined by The Diagnostic and Statistical Manual of Mental Disorders (DSM-5) (American Psychiatric Association, 2013) (American Psychiatric Association, 2013). Subjects were also excluded with current pregnancy, traumatic brain injury (TBI), meningitis, active implanted device, evidence or history of serious medical or neurological illness, such as cardiovascular, gastrointestinal, hepatic, renal, or other systemic illness; carotid atherosclerosis, cervical vagotomy or positive toxicology screen. Psychiatric diagnosis was evaluated with the Structured Clinical Interview for DSM (SCID) (First and Gibbon, 2004). The Clinician Administered PTSD Scale-5 (CAPS-5) was administered to evaluate for presence and severity of both current and lifetime PTSD (Blake et al., 1995; Weathers et al., 2018). The PTSD Checklist (PCL)-Civilian version was used to assess self-reported levels of PTSD symptoms (Ruggiero et al.,

2003). Anxiety was measured with the Hamilton Anxiety Scale (Ham-A) (Hamilton, 1959) and depression with the Hamilton Depression Scale (Ham-D) (Hamilton, 1960). Somatic anxiety was measured by adding the Ham-A items for “gastrointestinal” (Item 11, nausea, heartburn, abdominal pain) and “autonomic” (item 13, flushing, rapid heart rate, faintness, sweaty skin, dry mouth) (Maier et al., 1988). Clinical improvement was assessed by study personnel using the Clinical Global Impressions (CGI) scale, a 7 point scale ranging from 1 for very much improved, 2 much improved, 3 minimally improved to 4 for no change and 7 very much worse (Busner and Targum, 2007; NIMH, 1970). Among 64 individuals who were screened for eligibility, 20 were enrolled and randomized to active (N=9) or sham (N=11) stimulation. Pre-treatment inflammatory biomarker data was previously reported in three patients randomized to sham {Bremner, 2020 #11163}. One in the tcVNS group and three in the sham group dropped out after starting the protocol and follow-up assessments were not attainable (Figure 1). In the active tcVNS group, three (33%) met criteria for current co-morbid major depression and six (66%) for a lifetime history of major depression, four (44%) for current generalized anxiety disorder, one (11%) for current panic disorder with agoraphobia, two (22%) for current panic disorder without agoraphobia, two (22%) for current social phobia, and one (11%) for current body dysmorphic disorder. In the sham stimulation group, four (36%) met criteria for lifetime major depression, one (9%) for current major depression, three (27%) for current generalized anxiety disorder, and one (9%) for current obsessive-compulsive disorder.

## 2.2. Study Design

The participants provided their own traumatic experiences, and personalized voice recordings based on these experiences were presented as traumatic stress (Bremner et al., 1999; Orr et al., 1998). Subjects underwent exposure to personalized traumatic scripts in conjunction with tcVNS or sham on day 1, and “neutral” stressful tasks with tcVNS or sham on days 2 and 3 including public speech and mental arithmetic (Figure 2) (Bremner et al., 2009; Bremner et al., 2003; Burg and Soufer, 2014). We have described these paradigms in detail before and they have been shown to reliably produce behavioral and physiological responses consistent with a stress response (Bremner et al., 2009; Bremner et al., 2003; Hammadah et al., 2017b). The first day included six traumatic recall scripts (approximately one-minute each) and six neutral scripts presented audibly through headphones. The neutral scripts were designed to induce positive feelings to the subject, such as the description of pleasant scenery. Immediately after the traumatic stress recording ended, stimulation (active or sham) was applied by the researcher from the left side of the neck. Behavioral ratings after each task were performed using Visual Analogue Scales (VAS) rating subjective anger on a 0–100 scale with 100 being most extreme anger and 0 not at all (Southwick et al., 1993). On the same day two stimulation administrations (active or sham) were applied without any stressor. Blood draws were taken on the start of the day (baseline) and at multiple time points after. The second and third days were identical to each other. Baseline blood draws were taken both mornings. Afterwards, participants underwent a public speech task and mental arithmetic task, as previously described (Gurel et al., 2020b; Hammadah et al., 2017a). Participants were then instructed in use of the device for self-administration at home and received active or sham devices to take home. They were instructed to stimulate for two minutes on the left side, followed a one minute rest, and two minutes on the right

side, and to do this once in the morning and once at night. They were further instructed to stimulate while listening to personalized traumatic scripts twice a week. Participants continued twice daily stimulation for three months and returned for behavioral assessments once a month. At the end of the three month period they were given an active device and instructed to continue twice daily stimulation treatments.

### 2.3. Blinding

The participants were randomized into active tcVNS or sham groups with pre-numbered devices by the manufacturer who were not involved in the research. Random allocation was carried out by personnel who did not take part in data collection or analyses. The participants and researchers were blinded to the stimulus type. Statistical analyses were carried out by a biostatistician who did not take part in data collection or processing. Stimulus groups was un-blinded for the interpretation of statistical analysis.

### 2.4. Transcutaneous Cervical Vagal Nerve Stimulation

Both active tcVNS and sham stimuli were administered using hand-held devices that target the cervical portion of the vagus nerve from the skin (GammaCore, ElectroCore, Basking Ridge, New Jersey). Stimulation was applied using collar, stainless steel electrodes with a conductive electrode gel placed on the left side of the neck over the carotid sheath as determined by palpation of the carotid artery (Figure 3). Active tcVNS devices produced an alternating voltage signal consisting of five 5kHz sine bursts (1 ms of five sine waves with pulse width of 40 ms) repeating at a rate of 25 Hz envelopes. The frequency of 25 Hz was chosen based on prior studies showing optimization of effects on autonomic function and other measures at this frequency (Adair et al., 2020; Badran et al., 2019; Badran et al., 2018a; Badran et al., 2018b; Bikson et al., 2017a; Hays et al., 2014; Hays et al., 2013; Hulsey et al., 2017). The sham devices produce an alternating biphasic voltage signal consisting of 0.2 Hz square pulses (pulse width of 5 s) eliciting a mild sensation. The peak voltage amplitudes for active and sham device are 30V and 14V, respectively. An active stimulation amplitude higher than 15V using the studied device was previously reported to create vagal somatosensory evoked potentials associated with vagal afferent activation, that are also activated with VNS implants (Nonis et al., 2017). Both active and sham devices delivered two minutes of stimulation. The stimulation intensity (amplitude of the voltage wavefront) was adjustable using a roll switch that ranged from 0 to 5 a.u. (arbitrary units) with a corresponding peak output ranging from 0 to 30V for active tcVNS, and from 0 to 14 V for the sham device. During each application, the amplitude of the voltage waveform was increased to the maximum the subject could tolerate, without pain. The stimulation continued at the selected intensity.

The rationale behind the frequency difference between active (5kHz) and sham (0.2Hz) device waveforms is based on the fact that high frequency voltage signals (such as the active stimulus, 5kHz) pass through the skin with minimal power dissipation due to the low skin-electrode impedance at kHz frequencies. In contrast, lower frequency signals (such as the sham stimulus, 0.2Hz) are mainly attenuated at the skin-electrode interface due to the high impedance (Rosell et al., 1988). Accordingly, the active device operating at higher frequencies can deliver substantial energy to the vagus nerve to facilitate stimulation, while



the voltage levels appearing at the vagus would be expected to be orders of magnitude lower for the sham device and thus stimulation is unlikely. Nevertheless, since the sham device does deliver relatively high voltage levels directly to the skin, it activates skin nociceptors, causing a similar feeling to a pinch. This sensation is considered to be necessary for blinding of the participants, particularly longitudinal protocols such as in this manuscript.

## 2.5. Biomarker Assay

We employed the MesoScale system (MSD, Rockville, MD) multiplex assay to quantitate IL-6, IL-13, IL-22, IL-5, IL-12p70 and IFN- $\gamma$  in EDTA plasma. The MesoScale system (<https://www.mesoscale.com/>) was performed according to the protocols supplied by the manufacturer, and uses electrochemiluminescence for high sensitivity and broad dynamic range. Intra-assay CVs were 5.5% for IL-6.

**2.5.1. Statistical Analysis**—Analysis of variance (ANOVA) and chi-square tests were used to compare the demographic and retention characteristics across the tcVNS treatment or sham stimulation group among patients with PTSD. Independent t-tests were used to compare the demographic characteristics across the tcVNS treatment or sham stimulation groups. Paired t-tests were used to compare behavioral responses before and after treatment with  $p < 0.05$  denoting statistical significance. All statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, NC) and MATLAB (R2017b, Natick, MA).

## 3. Results

Participant groups were similar in age, body mass index, race, sex, education level and marital status (Table 1). There were no statistically significant differences in baseline PTSD symptom levels as measured by the CAPS or PCL, depression as measured by the Ham-D or anxiety as measured by the Ham-A. Participants in the tcVNS and sham stimulation groups experienced similar primary traumas, including one in each group with combat trauma, three in each group with childhood sexual abuse, two in each group with rape in adulthood, one in tcVNS and two in sham with physical assault in adulthood, two in tcVNS and one in sham with injury or death of someone close to them, and one in sham with a traumatic failed suicide attempt (Table 2).

TcVNS was associated with greater retention, 8/9 (89%) completing three months of treatment versus 8/11 (73%) in the sham stimulation group. These patients dropped out of the protocol and further assessments were not attainable. Active tcVNS resulted in a 31% greater decrease in PTSD symptoms measured with the PCL (pretreatment: 62 (14 SD); posttreatment: 51 (18 SD),  $p = .013$ , effect size .79) compared to sham stimulation (pretreatment: 59 (15 SD); posttreatment: 51 (20 SD),  $p = .08$ ) (Figure 4). There was a 31% decrease in PTSD symptoms on the CAPS in the tcVNS group (pretreatment: 46 (8 SD), posttreatment: 32 (17 SD)) versus a 23% decrease for sham (pretreatment: 38 (8 SD), posttreatment: 29 (11 SD),  $p < .05$  for both groups). tcVNS resulted in a 21% decrease in hyperarousal symptoms measured with the PCL (pretreatment: 19 (4 SD), posttreatment: 15 (5 SD),  $p = .008$ , effect size 1.0) versus a 17% decrease with sham stimulation (pretreatment: 20 (4 SD), posttreatment: 16 (6 SD),  $p = .06$ ) (Figure 5). tcVNS decreased overall anxiety as measured by the Ham-A in tcVNS (pretreatment: 23 (11 SD), posttreatment: 20 (9 SD),

$p=.10$ ) and sham stimulation groups (pretreatment: 21 (6 SD), posttreatment: 16 (10 SD),  $p=.09$ ). Active tcVNS resulted in a 46% decrease in somatic anxiety symptoms (autonomic/gastrointestinal) (pretreatment: 3.3 (2.4 SD), posttreatment: 1.8 (2.1 SD),  $p=.035$ , effect size .63) versus a 35% decrease with sham (pretreatment: 2.4 (1.9 SD), posttreatment: 1.6 (1.7 SD),  $p=.22$ ) (Figure 6). Treatment did not result in significant changes in depression as measured by the Ham-D in either the tcVNS (pretreatment: 19 (12 SD), posttreatment: 17 (8 SD),  $p=.53$ ) or sham stimulation groups (pretreatment: 18 (6 SD), posttreatment: 15 (8 SD),  $p=.23$ ). The CGI showed a pattern of greater improvement in tcVNS versus sham at one month (3.83 (1.33 SD) versus 4.00 (0.82)) and three months (3.29 (1.11 SD) versus 4.00 (0.82)) and significant improvement after three months of open label treatment following the double blind phase compared to three months of sham treatment (2.75 (0.71) versus 4.00 (0.82 SD) ( $p=0.003$ ) (intermediate between 2 for much improved and 3 for minimally improved (Figure 7). Both active tcVNS and sham stimulation were well tolerated and there were no adverse effects.

Exposure to personalized traumatic scripts in PTSD patients in conjunction with sham stimulation (but not tcVNS) resulted in a significant increase in IL-6 both pre-treatment (Figure 8) post-treatment (Figure 9). There were no statistically significant differences between tcVNS and sham stimulation groups in Interferon Gamma, IL-12, IL-13, IL22, or IL-5 (Supplementary Table).

#### 4. Discussion

Non-invasive tcVNS in this study was associated with a decrease in PTSD symptoms with the greatest effects on hyperarousal and autonomic or somatic anxiety symptoms. tcVNS also blocked the interleukin (IL)-6 response to traumatic script stress in PTSD patients. tcVNS was well tolerated and there were no adverse effects of administration over three months. tcVNS may be useful for some patients with PTSD, including those who do not respond to medication treatments and patients with increased symptoms of hyperarousal and/or autonomic imbalance or patterns of elevated inflammatory biomarkers.

These findings suggest that tcVNS may target the underlying neurobiology of PTSD, in particular noradrenergic and peripheral sympathetic nervous system function. The findings are consistent with our prior studies showing a decrease in sympathetic nervous system activity when tcVNS is paired with traumatic reminders in traumatized individuals (Gazi et al., 2020; Gurel et al., 2020a; Gurel et al., 2020b; Gurel et al., 2020e), as well as numerous studies showing nVNS (taVNS) blocks peripheral sympathetic nervous system function and startle reflex (Bretherton et al., 2019; Clancy et al., 2014; Lamb et al., 2017). Symptoms of hyperarousal reduced by tcVNS in this study include increased attention and vigilance, being on guard, poor concentration and sleep. Autonomic symptoms captured by the Ham-A that were reduced by tcVNS include those in the somatic anxiety categories of “gastrointestinal” (nausea, heartburn, abdominal pain) and “autonomic” (flushing, rapid heart rate, faintness, sweaty skin, dry mouth). These symptoms are known to be associated with increased peripheral sympathetic nervous system function.



A key role of VNS is modulation of norepinephrine (NE) centrally in the brain and peripherally through the sympathetic nervous system (Follesa et al., 2007; Krahl et al., 1998; Manta et al., 2009a, 2009b; Manta et al., 2013). VNS modulates NE in the brain through effects on the locus coeruleus (LC), an area in the brainstem where the majority of NE neurons are located (Hulsey et al., 2017). The vagus nerve has efferent fibers that project to the periphery and modulate organ function and afferent fibers that relay through the Nucleus Tractus Solitarius (NTS) in the brainstem to affect central brain function (Roosevelt et al., 2006; Ura et al., 2013). The NTS has inputs to the LC and VNS acts through the LC to increase NE release in key brain areas implicated in stress, emotion, and PTSD, including the medial prefrontal cortex, amygdala and hippocampus (Hassert et al., 2004; Hulsey et al., 2017; Manta et al., 2009a; Roosevelt et al., 2006). Increased NE has a secondary effect on neurochemical systems that have been the target of medication treatments for PTSD, including the serotonin (5HT) system. NE acts through excitatory alpha-1 adrenoreceptors on serotonergic neurons to increase 5HT in the dorsal raphe, the major site of serotonin cell bodies in the brainstem, with secondary effects on the same target brain regions modulated by NE (Manta et al., 2009a, 2009b; Manta et al., 2013; McGaugh, 1985). Animal studies show that chronic VNS treatment increases firing rates of both NE neurons in the LC and 5HT neurons in the dorsal raphe (Dorr and Debonnel, 2006), resulting in increased extracellular NE in the hippocampus and prefrontal cortex, and 5HT in the dorsal raphe (Manta et al., 2009a; Manta et al., 2013; Nichols et al., 2011). Chronic VNS treatment increases metabolites of dopamine and 5HT in the cerebrospinal fluid (CSF) in patients with epilepsy (Hammond et al., 1992). VNS acts through these central brain areas in ways that are incompletely understood to decrease peripheral sympathetic function and enhance parasympathetic function (Brock et al., 2017; Clancy et al., 2014; Hammond et al., 1992; Pagani et al., 1986; Thayer and Lane, 2007; Weber et al., 2010). This fits with our findings that tcVNS blocks peripheral sympathetic activation and enhances parasympathetic tone (Gurel et al., 2020b).

Alterations in noradrenergic and peripheral sympathetic function play an important role in the maintenance of symptoms of PTSD (Bremner et al., 1996a, 2009b; Southwick et al., 1997). These systems represent key components of the stress response that ready the body to prepare to deal with potential threat (Bremner et al., 1996a, 1996b). NE cell bodies in the LC have axons that extend throughout the rest of the brain and are activated by stress, resulting in release of NE in the brain with associated increased attention, fear, and anxiety behaviors, as well as activation of the peripheral sympathetic system with increased heart rate, blood pressure, and respiration (Abercrombie and Jacobs, 1987a, 1987b; Aston-Jones et al., 1991; Foote et al., 1983; Jedema et al., 2001; Levine et al., 1990; Nisenbaum and Abercrombie, 1993; Redmond and Huang, 1979). Chronically stressed animals re-exposed to stress show a potentiated release of NE in brain areas involved in emotion and the stress response which is associated with anxiety like behaviors (Aston-Jones et al., 1991; Finlay et al., 1995; Miner et al., 2006; Nisenbaum et al., 1991; Petty et al., 1993; Tanaka et al., 2000; Torda et al., 1984; Weiss et al., 1981). Multiple lines of evidence support increased noradrenergic function in PTSD, including the fact that drugs of abuse and medications that inhibit LC firing reduce symptoms of hyperarousal, including opioids (Bremner et al., 1996c), and agonists of the  $\alpha_2$  NE inhibitory autoreceptor on the LC, such as clonidine

(Kinzie and Leung, 1989), while  $\alpha_2$  NE antagonists, like yohimbine, have the opposite effect (Southwick et al., 1997; Southwick et al., 1993). Other studies in PTSD found increased peripheral concentrations of NE and its metabolites in urine and plasma (De Bellis et al., 1999; De Bellis et al., 1994; Lemieux and Coe, 1995; Mason et al., 1988; Yehuda et al., 1998) as well as cerebrospinal fluid at baseline (Geraciotti et al., 2001). In PTSD patients, exposure to traumatic reminders increased symptoms of PTSD and increased NE and its metabolites, in addition to increasing heart rate, blood pressure, and skin conductance (Blanchard et al., 1986; Blanchard et al., 1982; Blanchard et al., 1991; Malloy et al., 1983; McFall et al., 1990; McFall et al., 1992; Orr et al., 1998; Orr et al., 1995; Orr et al., 1993; Orr and Roth, 2000; Shalev et al., 1998). Challenge to the NE system of patients with PTSD with the  $\alpha_2$  adrenergic receptor antagonist, yohimbine, had similar effects (Bremner et al., 1997; Southwick et al., 1997; Southwick et al., 1993). These findings show that altered NE and sympathetic system function play an important role in PTSD symptoms, especially in the hyperarousal category, highlighting the potential utility of interventions such as tcVNS that modulate NE and block peripheral sympathetic function.

The current study is a partial replication of our prior report on its effects on IL-6 response to traumatic script stress in PTSD (Bremner et al., 2020a). IL-6 is linked to autonomic function, so it makes sense that VNS has effects on peripheral inflammation (Jan et al., 2010; Marsland et al., 2007). Our findings add to the growing literature that VNS blocks inflammatory and autonomic responses in human (Frangos et al., 2015; Frangos and Komisaruk, 2017; Gurel et al., 2020b; Gurel et al., 2020e;; Lerman et al., 2019; Lerman et al., 2016; Yakunina et al., 2017) and animal studies (Brock et al., 2017; Chen et al., 2016; Oshinsky et al., 2014). Increased inflammatory function is increasing seen as being a response to stress and playing a role in stress-related psychiatric disorders like PTSD and depression (Miller et al., 2009; Miller and Raison, 2016; Raison and Miller, 2013). Studies in both animals and humans showed that catecholamines released during stress act through the adrenergic receptor to activate the transcription factor, nuclear factor- $\kappa$ B (NF- $\kappa$ B), which leads to increases in cytokines, including IL-6 (Bierhaus et al., 2003). Intervention at the level of IL-6 with VNS may be a useful intervention that reduces symptoms by targeting the underlying neurobiology of PTSD (Bremner et al., 2020b; Noble et al., 2019; Souza et al., 2019).

This is a pilot study with a small sample size that needs to be replicated with larger numbers of patients. The small sample size and multiple outcome measures could lead to spurious results. We also had imperfect follow-up, which is consistent with the highly symptomatic nature of the PTSD patients in this study. Future studies need to be performed with larger numbers of patients to replicate and extend these results.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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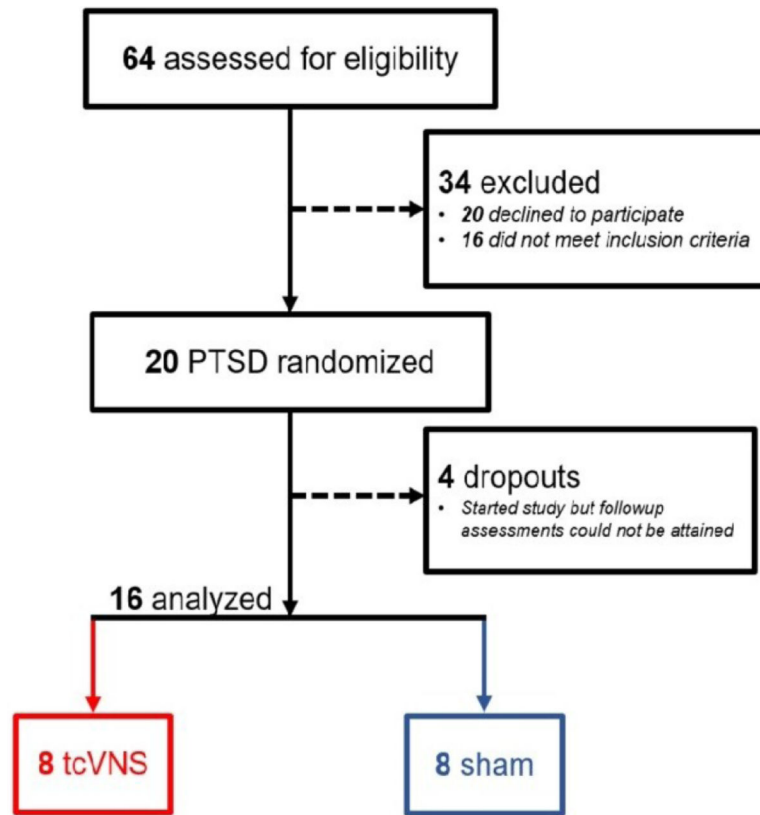
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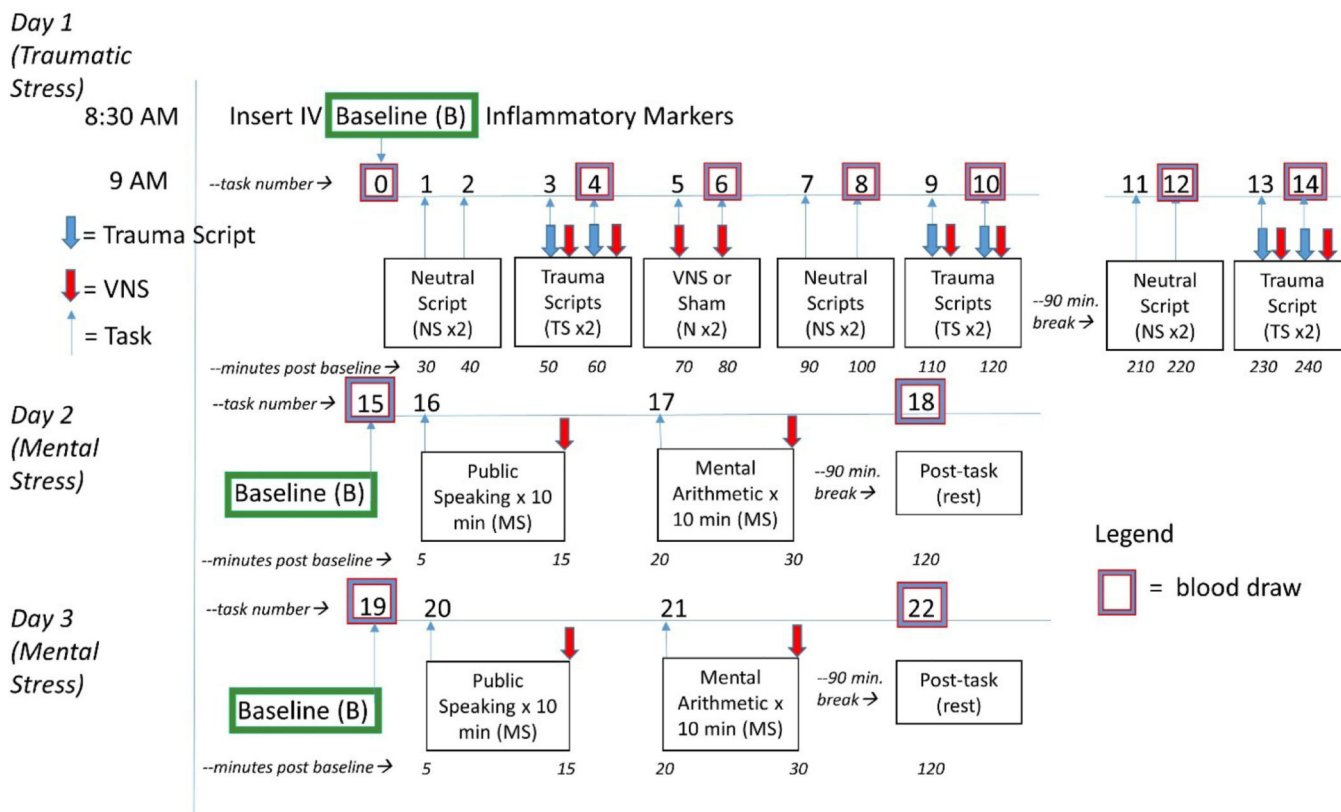
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**Figure 1.** CONSORT diagram showing flow of study participants screened, enrolled, and completing the protocol.



**Figure 2.** Diagram of the baseline study protocol. PTSD patients underwent three days of stress, one day (Day 1) with neutral scripts (NS) and personalized traumatic scripts (TS), and two days (Days 2 and 3) with mental stress (MS) involving public speaking and mental arithmetic tasks. Participants underwent randomized, double-blind assignment to tcVNS or sham stimulation which was paired with stress tasks (or no task) on Days 1, 2 and 3. On Day 1 neutral and traumatic scripts lasted about one minute and occurred in pairs with 10 minutes in between. Stress tasks were paired with stimulation with tcVNS or sham which began immediately after termination of the task and continued for two minutes followed by a blood draw (purple/blue boxes signify pairing of task/stimulation/blood draw but blood draw actually occurred at the termination of stimulation). On Day 1 participants also underwent stimulation with tcVNS or sham for two minutes in the absence of a task (N) repeated twice with 10 minutes in between followed by a blood draw. Neutral and traumatic script pairs were repeated followed by a 60 minute rest and lunch break, with a repeat of neutral and traumatic script pairs in the afternoon each paired with blood draws. The neutral scripts tasks #11 and #12 were followed by a blood draw (which was about 110 minutes after the first trauma script pairs at tasks #3 and #4) and the trauma scripts tasks #13 and #14 paired with tcVNS or sham were followed by the final blood draw at 210 minutes into Day 1 (Traumatic Stress). On Day 2 after a baseline blood draw at rest (task #15) participants underwent mental stress (MS) involving five minutes of public speaking (task #16) with tcVNS or sham at the end, followed by an eight minute rest period, and another five minutes of mental arithmetic (task #17) followed by tcVNS or sham. After a 90 minute rest period participants underwent a blood draw at rest (task#18). This was repeated for Day 3 with

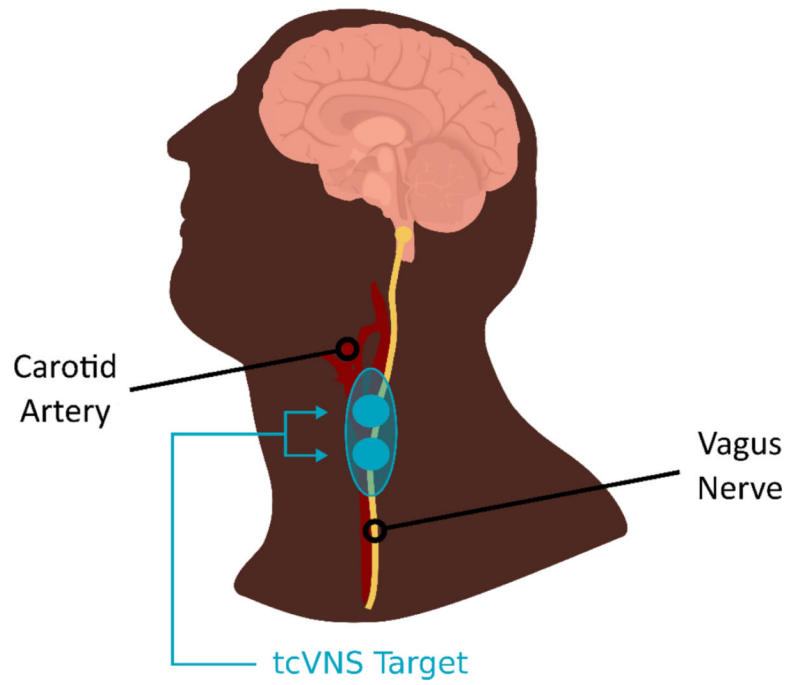
baseline (task #19, public speaking (task #20), mental arithmetic (task #21) and a blood draw post-task at rest (task #22). The blood draws for all three days were timed to coincide with the roughly 90 minute time course of interleukin-6 (IL-6) response to stress based on prior studies. Patients then underwent three months of tcVNS/sham followed by a repeat of Day 1 only.

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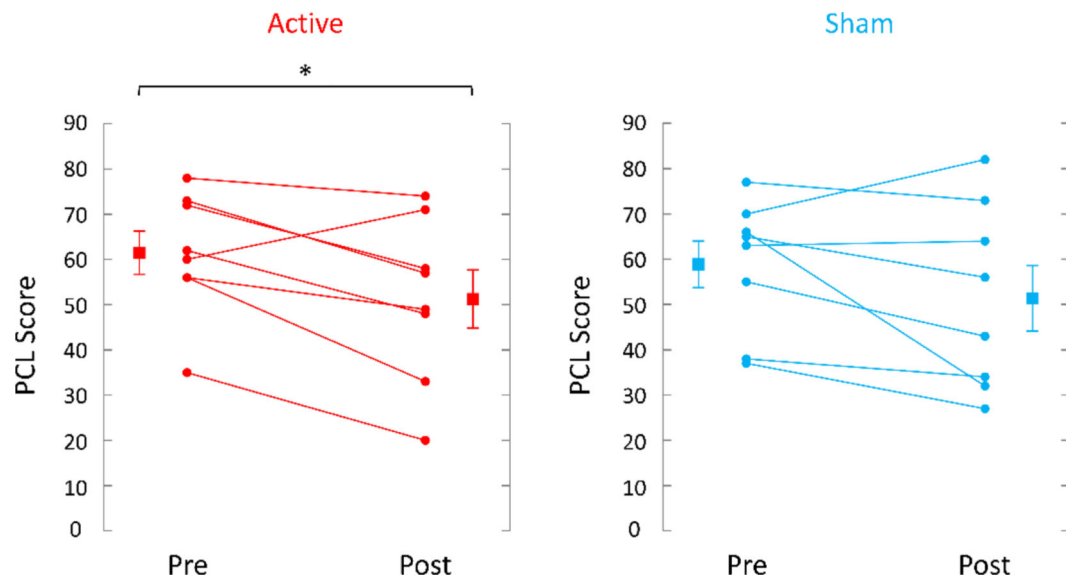
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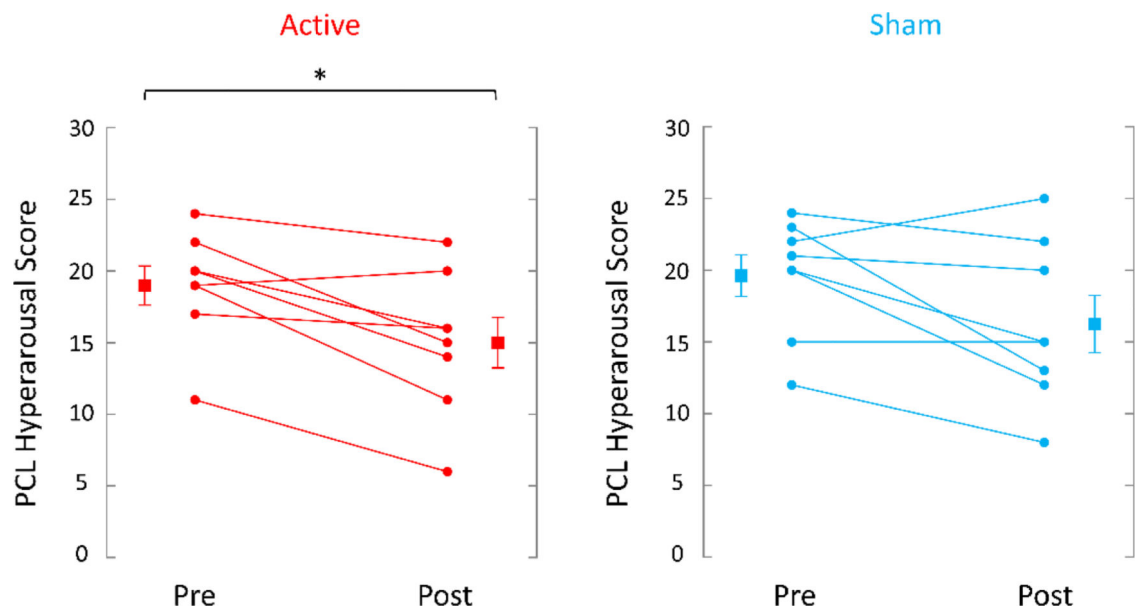


**Figure 3.** Diagram showing placement of tcVNS device on the neck to target the vagus nerve as it travels through the carotid sheath.



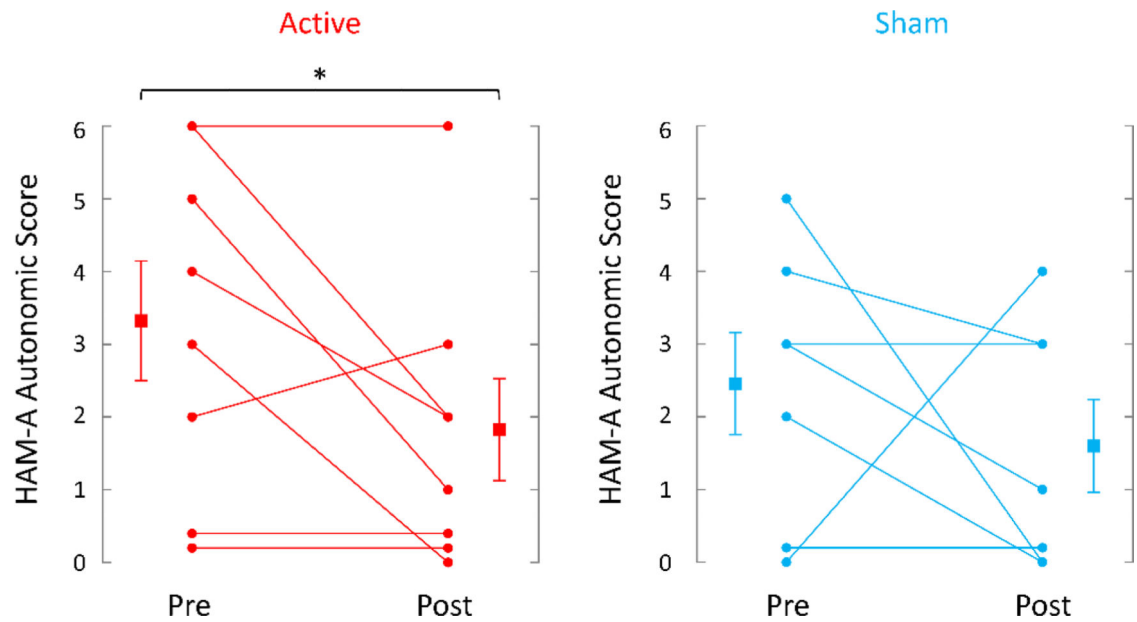
**Figure 4.** Effects of up to three months of twice daily transcutaneous cervical Vagus Nerve Stimulation (tcVNS) (on the left, red lines) or sham stimulation (on the right, blue lines) on symptoms of PTSD as measured with the PTSD Checklist (PCL). Individual participants are shown with lines separating pre- and post-treatment; lines with bars represent means and SD before and after treatment for both groups. Active tcVNS resulted in a 17% reduction in PTSD symptoms ( $p=.013$ ) and sham stimulation a 13% reduction in PTSD symptoms after treatment ( $p=.15$ ) ( $*p<.05$  from pretreatment).





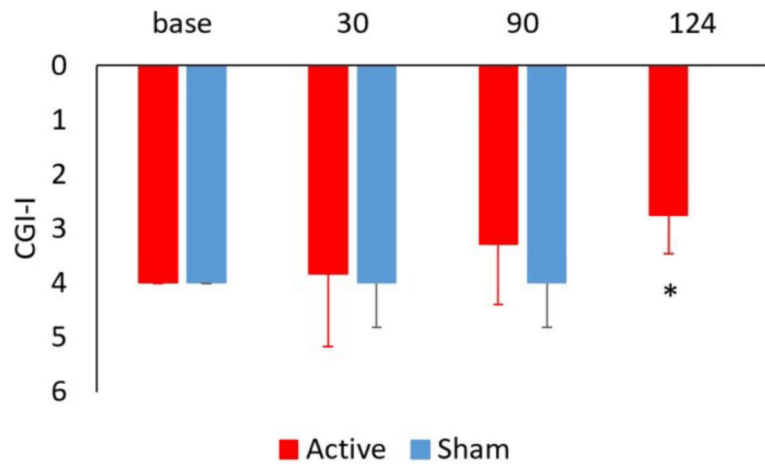
**Figure 5.**

Effects of up to three months of twice daily tcVNS (on the left, red lines) or sham stimulation (on the right, blue lines) on symptoms of PTSD as measured with the PTSD Checklist (PCL). Individual participants are shown with lines separating pre- and post-treatment; lines with bars represent means and SD before and after treatment for both groups. Active tcVNS resulted in a 21% reduction in hyperarousal symptoms ( $p=.008$ ) while sham stimulation resulted in a 17% decrease ( $p=.06$ ) ( $*p<.05$  from pretreatment).



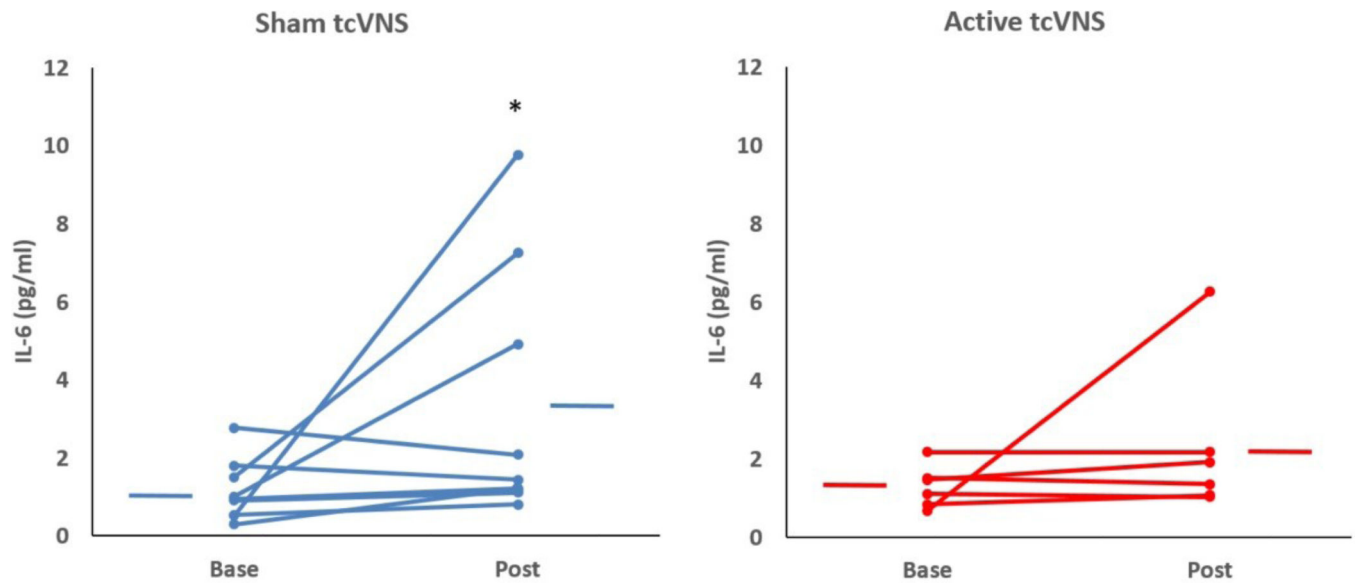
**Figure 6.**

Effects of tcVNS and sham on autonomic anxiety as measured with the Hamilton Anxiety Scale (Ham-A). Scores represent the sum of items for gastrointestinal and autonomic somatic anxiety (see text) at baseline and with three months of twice daily tcVNS (on the left, red lines) or sham stimulation (on the right, blue lines) on autonomic anxiety as measured with the Ham-A. Individual participants are shown with lines separating pre- and post-treatment; lines with bars represent means and SD before and after treatment for both groups. There was a  $-46\%$  decrease in Ham-A somatic anxiety in the tcVNS group ( $p=.036$ ) versus a  $-35\%$  change in the sham stimulation group ( $p=.22$ ) ( $*p<.05$  from pretreatment).

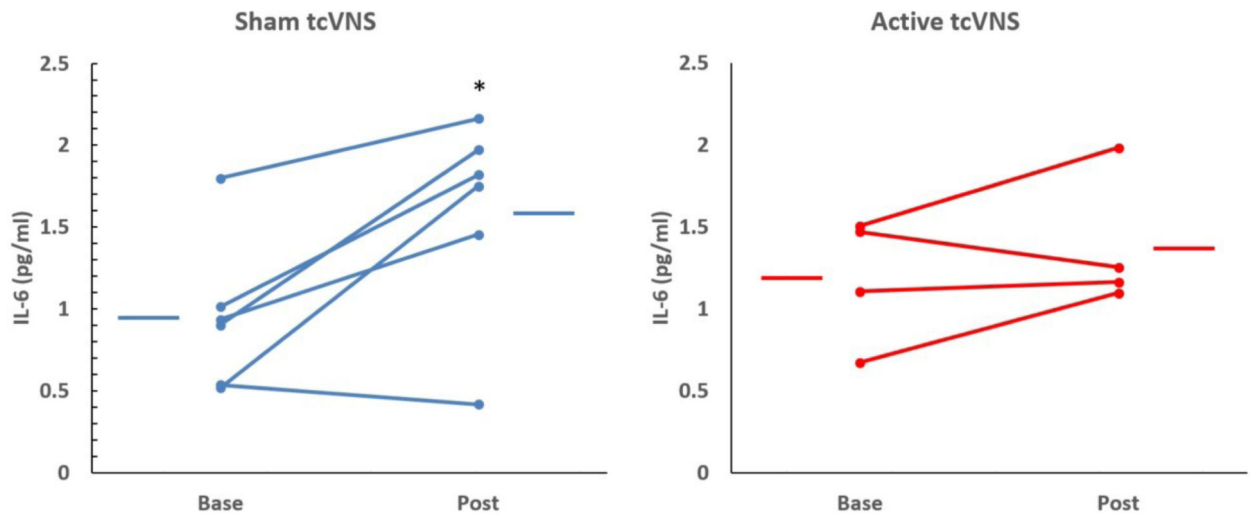


**Figure 7.**

Effects of tcVNS and sham on clinical improvement as measured with the Clinical Global Impressions scale-improvements (CGI-I) in active tcVNS (red) and sham (blue) stimulation groups at baseline and 30 and 90 days after start of double-blind active (N=9) versus sham (N=11) treatment in patients with PTSD. The final measurement at 124 days (34 days after start of open label treatment) showed a significant improvement compared to after three months of sham stimulation (2.75 (0.71) versus 4.00 (0.82 SD) (\*p=0.003). This score is intermediated between much improved (2) and minimally improved (3) compared to no improvement (4).



**Figure 8.** Effects of tcVNS (red lines, right side) or sham (blue lines, left side) on interleukin-6 (IL-6) at baseline (base) and following repeated exposure to traumatic script stress (post) in patients with PTSD. Lines connect pre and post stress in individual patients and bars represent the means for each group. There was a significant increase in IL-6 in the sham group (\* $p < 0.05$ ) not seen in the PTSD group.



**Figure 9.** Effects of tcVNS (red lines, right side) or sham (blue lines, left side) on interleukin-6 (IL-6) at baseline (base) and following three months of double blind active tcVNS or sham treatment in patients with PTSD. Lines connect baseline to post-treatment and post traumatic script stress in 5/9 patients and bars represent the means for each group. There was a significant increase in IL-6 in the sham group (\* $p < 0.05$ ) not seen in the PTSD group.

**Table 1**

Baseline Demographic and Behavioral Variables in Active tcVNS and Sham Stimulation Groups

	tcVNS (n=9)	Sham (n=10)
<b>Age</b>		
Mean ± SD	37 ± 13	40 ± 14
<b>Race</b>		
White	5 (55%)	5 (45%)
Black	4 (45%)	5 (45%)
Asian/Pacific Islander	0 (0%)	1 (9%)
<b>Sex</b>		
Female	6 (66%)	7 (64%)
Male	3 (33%)	4 (36%)
<b>BMI</b>		
Mean ± SD	30 ± 7	30 ± 6
<b>Education Level</b>		
Some high school	1 (11%)	1 (9%)
High school graduate	3 (33%)	1 (9%)
Some college	2 (22%)	2 (18%)
College graduate	3 (33%)	7 (64%)
<b>Marital Status</b>		
Never married	3 (33%)	5 (45%)
Married	3 (33%)	2 (18%)
Divorced / Separated	2 (22%)	3 (27%)
Widowed	1 (11%)	1 (9%)
<b>PTSD Score (PCL)</b>		
Mean ± SD	62 ± 14	61 ± 13
<b>CAPS Score</b>		
Intrusions-Mean ± SD	11 ± 2	10 ± 3
Avoidance-Mean ± SD	5 ± 2	5 ± 1
Negative Cognitions-Mean ± SD	17 ± 4	14 ± 4
Hyperarousal-Mean ± SD	11 ± 3	10 ± 3
Total-Mean ± SD	44 ± 9	38 ± 9
<b>Ham-D Score</b>		
Mean ± SD	19 ± 12	19 ± 5
<b>Ham-A Score</b>		
Mean ± SD	23 ± 11	20 ± 6

tcVNS=transcutaneous Vagal Nerve Stimulation; sham=sham stimulation; BMI=body mass index; PCL=PTSD Checklist; CAPS=Clinician Administered PTSD Scale; Ham-D=Hamilton Depression Scale; Ham-A=Hamilton Anxiety Scale



**Table 2**

## Key Traumatic Events in Active tcVNS and Sham Stimulation Groups

Subject/group/sex	Traumatic Event
001 sham female	Husband left for hockey game, never returned
002 active male	Iraq combat
003 active female	Childhood sexual abuse from age 7
004 sham male	Shot, saw friend shot and killed
005 active female	Sudden death of husband, received news while driving, almost crashed
006 sham female	Sexual abuse by father from age 6
007 active female	Gang rape in adulthood
008 sham female	Childhood sexual abuse
009 active female	Raped in childhood, held at knifepoint.
010 sham female	Raped at knifepoint at age 14.
011 sham male	Failed suicide.
012 active male	Childhood sexual abuse. Raised in religious cult.
013 active female	Mother attempted suicide multiple times in childhood.
014 sham female	Rape in adulthood. Physical and emotional abuse in childhood.
015 sham male	Vietnam combat
016 sham female	Child sexual abuse and assault, adult rape
017 active female	Kidnapped and assaulted, abusive relationship
018 active male	Stabbed twice in military, almost died
019 sham male	Physical assault in military, stabbed in a fight
020 sham female	Rape, parents in cult as child